OCT 21 1936

VOLUME LIX

NUMBER 3

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

September, 1936

Diffuse Aneurysmal Dilatation of the Pulmonary Artery and Both of Its Branches. SIDNEY W. JENNES 133

Primary Pulmonary Arteriosclerosis. Report of a Case with Marked Calcification of the Pulmonary Arteries. ROBERT F. NORRIS, M.D. 143

Severe Myocarditis of Unknown Etiology. HELEN B. TAUSSIG AND ELLA H. OPPENHEIMER 155

The Mechanism of a Hitherto Unexplained Form of Native Immunity to the Type III Pneumococcus. ARNOLD R. RICH AND CLARA M. MCKEE 171

Book Reviews 209

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore, Maryland

Made in United States of America

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, JAMES BORDLEY, III

EDITORIAL BOARD

E. Cowles Andrus
Charles R. Austrian
Thomas R. Boggs
Chalmers L. Gemmill
Arnold R. Rich
Frank R. Smith

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefore adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire, except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

THE JOHNS HOPKINS PRESS
BALTIMORE, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918

DIFFUSE ANEURYSMAL DILATATION OF THE PULMONARY ARTERY AND BOTH OF ITS BRANCHES

SIDNEY W. JENNES

From the Medical Clinic, School of Medicine, The Johns Hopkins University

Submitted for publication July 8, 1936

Aneurysmal dilatation of the pulmonary artery is a condition which has been considered extremely rare, especially if we compare its incidence with that of aneurysm of the aorta which is relatively common. The condition we are describing, that of diffuse aneurysmal dilatation of the pulmonary artery and of both its branches, is of even greater rarity. It is for that reason, as well as for the infrequency of its clinical recognition, that we are presenting these two cases, one of which Dr. John King and the author have been following for about eight months, and the other in a patient who was seen here in 1934, although the true nature of the underlying condition was not recognized at the time.

The relative infrequency of aneurysmal dilatation of the pulmonary artery can be realized by a study of autopsy records. In an examination we made of those of the Johns Hopkins Hospital no instance was found in 14,523 autopsies performed at this hospital to the present day. According to Scott (8), none was found in 13,234 autopsies done at St. Bartholomew's Hospital between 1867 and 1912. In the records of the city of New York there was none in 10,000 autopsies performed between 1918 and 1924. Crisp found two in a study of 530 aneurysms.

D'Aunoy and Von Haam (3), in 1934, list 85 cases of aneurysm of the pulmonary artery, which they collected from the literature up to 1934. To these they add two cases of their own. Oppenheimer (7), in 1933, published a report of 8 cases of what he called "idiopathic dilatation of the pulmonary artery," which seem to fall properly into the group we are describing.

Guénard and Caubet (4) in 1933, report a case, one of the few clinically diagnosed. Scott, in 1934, adds his own case and two from the literature that D'Aunoy and Von Haam had overlooked. Joules (5),

also, in 1934, reported another case. Luisada (6) in the same year refers to 15 cases not elsewhere described, plus a case of his own. Brenner (1), in 1935, in a discussion of diseases of the pulmonary artery refers to 23 cases of pulmonary aneurysm. Of six of these we can find no mention in any of the other reports. Thus, we have a total of 122 up to this present report. So far as we have been able to determine, all of them, with the exception of twelve, were recognized only at necropsy.

The *first patient* was L. M., a 47-year-old negress, first seen in the dispensary of this hospital on August 19, 1933, with the complaint of "misery" in the chest.

The family history is non-contributory. The patient had been married twenty-three years. She had had nine pregnancies, with one miscarriage previous to her last pregnancy, which was nine years before she came to us. Her general health had always been good. There was no cyanosis or dyspnea. She gave an indefinite history of somewhat more easy fatigability than other children, and of vague pains in her knees about the time she was married—not typically rheumatic in character. The rest of the past history was essentially negative. The patient had had her menopause at forty years of age.

The onset of the *present illness* dated from January 1935, when she first began to feel tired and to suffer from "misery" in her chest. She also suffered from some shortness of breath. This was not constant, but would occur more in the form of attacks lasting for several days. She found it impossible to carry on her work as well as formerly. In April, she had had a particularly severe attack which put her to bed for several days. Since then she had done practically no work. In the early part of August she had had her most severe attack, which had continued up to the time of her visit to the dispensary. The "misery" in her chest was almost constant, and she had to rest most of the time.

The following are the essential findings on physical examination. The patient was a poorly developed and nourished 47-year-old colored female. The only important finding in the head was the ophthalmoscopic examination which showed only slight A-V nicking, with no tortuosity of the arteries. The fundi were otherwise negative. There was a marked tracheal tug. A definite pulsation was visible in the suprasternal notch. The chest was asymmetrical with a marked cardiac bulge. The breasts were atrophic. There was an accessory nipple on each side. The lungs were negative except for the presence of moist râles at the right base. Examination of the heart revealed a forceful apex impulse that could be seen and felt in the sixth interspace, where there was also an expansile pulsation. Percussion revealed a tremendously enlarged heart, the significance of which can be better appreciated from the accompanying roentgenological plate. On auscultation a long, harsh, rough systolic murmur could be heard loudest at the third left interspace, transmitted to the neck and to the left mid-scapular

region. There was also a soft, short, higher-pitched diastolic murmur heard here, and over the pulmonic area. P_2 was snapping. The aortic sounds were normal, as were the mitral, where the systolic murmur was also audible, but with much less intensity. The radial arteries were slightly thickened. The pulses were regular and equal. Blood pressure 115/76 (left arm), 108/66 (right arm).

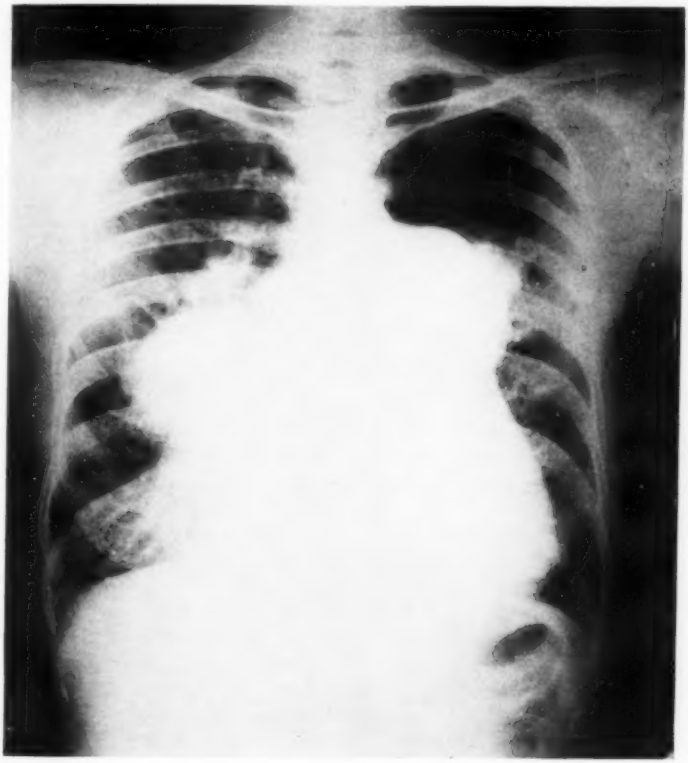


FIG. 1. CASE 1. ANTERIOR-POSTERIOR VIEW, SHOWING GREATLY DILATED PULMONARY CONUS AND RIGHT BRANCH OF THE PULMONARY ARTERY

The abdomen was negative. There was no peripheral edema of the extremities. No cyanosis or clubbing of the fingers.

The patient has remained in a fair state of cardiac compensation to the present date with the exception of one episode in the hospital.

Three Wassermann reactions were negative. There was no polycythemia. Other blood examinations, including blood-chemistry tests, were negative. Urinalyses were also negative.

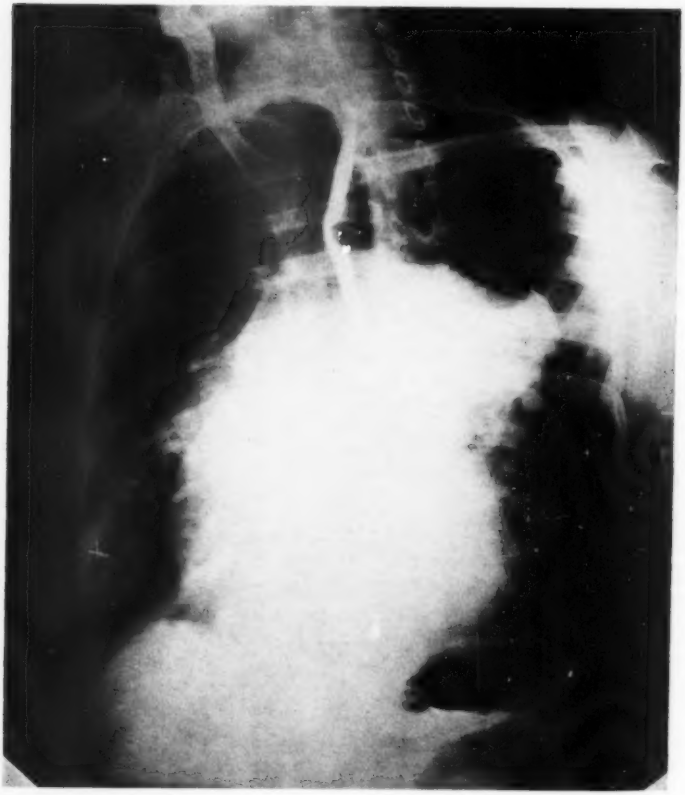


FIG. 2. CASE 1. LEFT OBLIQUE VIEW. BARIUM IN THE ESOPHAGUS. WEDGE-SHAPED SHADOW OF LARGE LEFT BRANCH OF THE PULMONARY ARTERY

The teleroentgenogram in the A-P view showed an enlarged heart with an enormous pulmonary conus and a large hilar shadow in the right lung, both of which were seen to pulsate on fluoroscopic examination. In both oblique views,

there could be noted wedge-shaped shadows, projecting posteriorly, which also pulsated and which apparently represented the right and left branches of the pulmonary artery (figures 1, 2, 3).

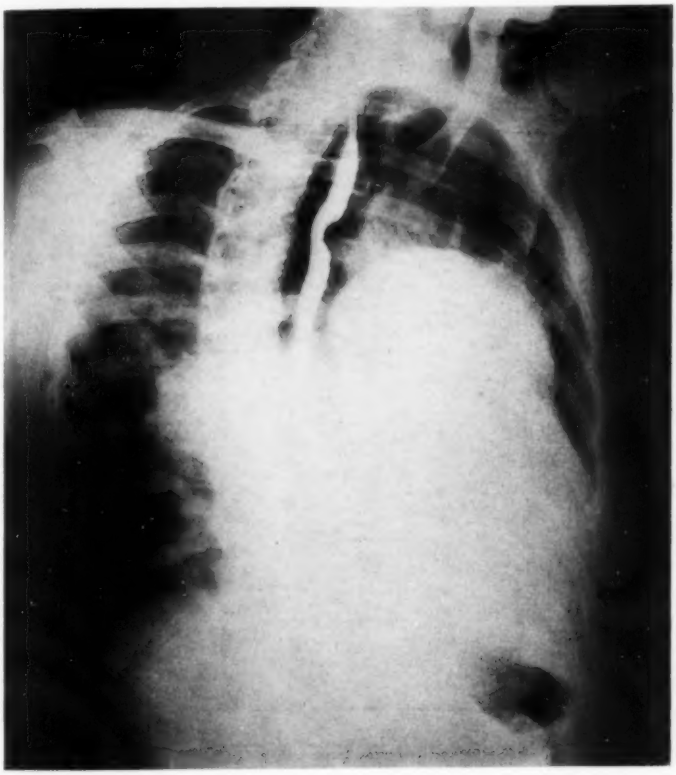


FIG. 3. CASE 1. RIGHT OBLIQUE VIEW. BARIUM IN ESOPHAGUS. DILATED RIGHT BRANCH OF THE PULMONARY ARTERY IS VISIBLE

The electrocardiogram showed the presence of a right ventricular preponderance (figure 4).

Impression: Congenital heart disease with patent interventricular septum, with the defect probably high up in the septum. Pulmonary regurgitation. Diffuse aneurysmal dilatation of the pulmonary arteries.

The *second patient* was R. B., who was seen at this hospital Nov. 1, 1934, with the chief complaint of oppression in the chest, cough and shortness of breath on exertion for ten days previous to his visit to the dispensary.

The family history was irrelevant. The patient's general health had always been good. There was no cyanosis or dyspnea when he was young. He had had moderate dyspnea since 1923.

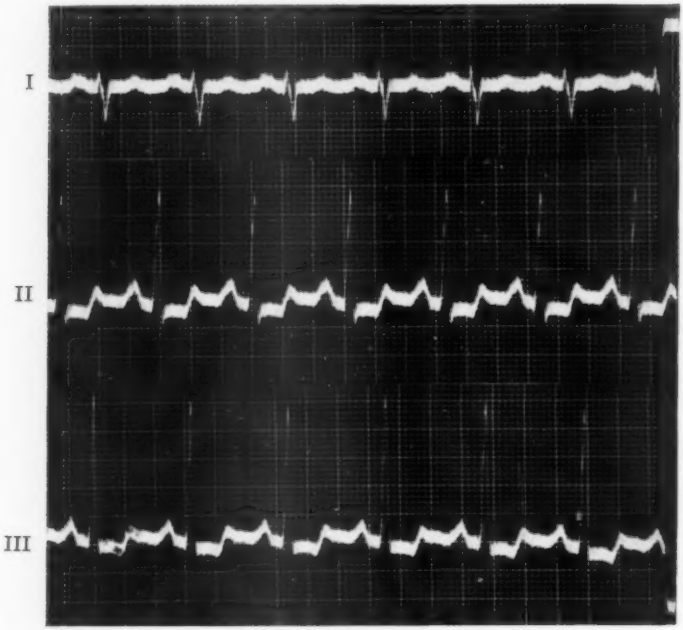


FIG. 4. CASE 1. ELECTROCARDIOGRAM. SHOWING RIGHT VENTRICULAR PREPONDERANCE. PATIENT WAS TAKING DIGITALIS

His *present illness* began ten to twelve days before his visit to the dispensary when he had a feeling as if his chest were clogged up. He would have a coughing spell for a minute or so in the morning, unproductive in character, as a rule. He had been noting that he had been losing his breath on the slightest exertion.

Physical examination revealed the following findings. Head and neck essentially negative. Chest slightly emphysematous in shape. There was a greater fullness over the left anterior chest than over the right. Expansion was limited on both sides. The breath sounds were harsh, with numerous râles of the type

heard in asthmatic bronchitis. A definite pulsation, with a diffuse impulse was noted over the heart in the second left interspace. No definite thrill was felt. There was a diastolic shock, especially, at the base. There was an inconstant

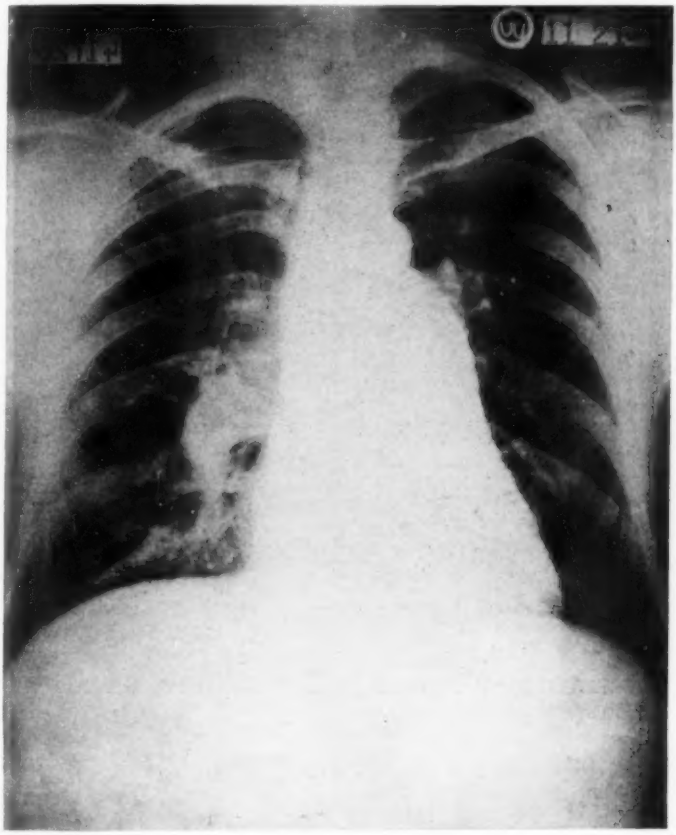


FIG. 5. CASE. 2. ANTERIOR-POSTERIOR VIEW. SHOWING DILATED PULMONARY CONUS AND DILATED RIGHT BRANCH OF THE PULMONARY ARTERY

systolic murmur at the apex, which was also audible over the pulmonic area and posteriorly at the angle of the scapula. No thickening of the radials was noted. The pulses were regular, with normal volume. The abdomen was negative. The extremities showed slight cyanosis, but no clubbing.

Urinalyses were essentially negative. The Wassermann reaction was negative.

Teleroentgenogram report: Marked accentuation of the pulmonary conus on the left side with perfectly enormous dilatation of the pulmonary artery. Heart moderately enlarged (see figure 5).

Electrocardiogram: Right ventricular preponderance (see figure 6).

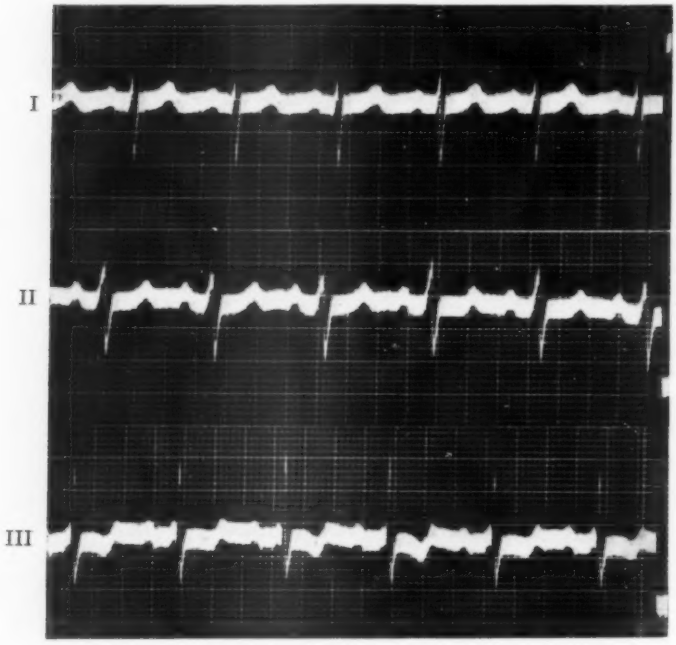


FIG. 6. CASE 2. ELECTROCARDIOGRAM. SHOWING RIGHT VENTRICULAR PREPONDERANCE

The impression at the time was that this was a case of congenital heart disease with dilatation of the pulmonary artery and its branches, and enlargement of the right heart. The question of the importance of the emphysema was considered.

Subsequently, the patient was admitted to the Pennsylvania Hospital, where he died of primary carcinoma of the liver.[1]

[1] A personal communication from the Pennsylvania Hospital informs us that Dr. Robert F. Norris will probably report the necropsy findings in this case.

DISCUSSION

So far as could be ascertained there have been a total of 122 cases previously described in the various literatures. Of these cases, the causes as listed are varied. In 12, sclerosis was considered the causative agent. In 33 syphilis was regarded as the factor—either on the basis of actual syphilitic disease of the pulmonary artery, on a positive Wassermann reaction, or on the presence of syphilitic disease elsewhere. In 4 cases the aneurysms were due to direct trauma. In 6 infection was held to be the etiological factor.

In 40 cases the condition was considered to be purely on a congenital basis. In addition, aside from a congenital lesion of the heart, in 10 more there was an associated infection, localized to the pulmonary artery, in 4 there was an associated syphilis, and in one an associated syphilis and arteriosclerosis. In 13 cases no cause was given.

An analysis of the 122 cases reported prior to ours discloses in only 11 a condition similar to that which our cases present—an involvement of the main pulmonary artery and of both its branches. In the first of these cases described in the literature, that of Zuber (12), in 1904, the case to which Oppenheimer referred, there is described an enormous aneurysmal dilatation of the pulmonary artery, fusiform in shape, together with aneurysmal dilatations of both the right and left branches.

Of Oppenheimer's eight cases, three out of the four patients who died were autopsied. These three cases exhibited tremendous dilatation of the pulmonary artery and all its branches.

In Scott's case, postmortem examination disclosed a widely dilated pulmonary artery with a saccular aneurysm of the left branch and an ovoid aneurysm of the right branch.

Joule reports in his case the presence of dilatation of the pulmonary artery with the right branch much larger than the descending aorta, and the left branch only slightly smaller.

In the other 94 cases, the aneurysms were localized, involving either the main pulmonary artery or one of the branches, or else the main artery and one branch. These aneurysms were variable in their dimensions. Some were quite small—about the size of a pea; others varied from this size to that of large saccular or fusiform aneurysms.

So far as can be discovered, only 12 such cases have been clinically recognized, the rest being discovered at autopsy. Amongst these 12 were Oppenheimer's 8 cases, 2 of the 46 cases collected by Henschen, the case of Joules and that of Guénard and Caubet. Of these patients only the last one and 4 belonging to Oppenheimer's series are still living.

A discussion of the etiology in these two cases reported here will not be given except to enumerate the possibilities which should be considered. They are (1) Patent ductus arteriosus. (2) Congenital defect of the wall of the artery. (3) An unequal division of the truncus arteriosus. (4) Sclerosis—either arteriosclerosis or primary pulmonary sclerosis. (5) Infection. (6) Syphilis.

SUMMARY

The clinical aspects of two cases of diffuse aneurysmal dilatation of the main pulmonary artery and both its branches, recognized during life, have been presented. In addition, a discussion of the condition has been given, with a brief analysis of the previously reported cases.

REFERENCES

(1) Brenner, O.: *Arch. Int. Med.*, **56:** 211, Sept., 1935.
(2) Cautley, E.: *Proc. Roy. Soc. Med.*, 1908–1909.
(3) D'Aunoy, R. and Von Haam, E.: *J. Path. & Bact.*, **38:** 39–60, Jan., 1934.
(4) Guenard, F. and Caubet: *Arch. d. mal. du coeur*, **26:** 261–266, Apr., 1933.
(5) Joules, H.: *Lancet*, **2:** 1338–1340, Dec. 15, 1934.
(6) Luisada, A.: *Minerva Med.*, **2:** 421–430, Sept. 29, 1934.
(7) Oppenheimer, B. S.: *Trans. A. Am. Physicians*, **48:** 290–297, 1933.
(8) Scott, R. B.: *Lancet*, **1:** 567–568, March 17, 1934.
(9) Sutherland, G. A.: *Brit. J. Childh. Dis.*, **20:** 27, 1923.
(10) Warthin, A. S.: *Am. J. Syph.*, **1:** 63, Oct., 1917.
(11) White, P. D., and Marble, H. C.: *J. A. M. A.*, **74:** 1778, 1920.
(12) Zuber, B.: *Jahrb. für Kinderh.*, **59:** 30–53, 1904.

PRIMARY PULMONARY ARTERIOSCLEROSIS

Report of a Case with Marked Calcification of the Pulmonary Arteries

ROBERT F. NORRIS, M.D.

From the Ayer Clinical Laboratory, The Pennsylvania Hospital, Philadelphia, Pennsylvania

Submitted for publication July 8, 1936

Numerous case reports of primary pulmonary arteriosclerosis have appeared in the literature. MacCallum (1) gave an extensive bibliography in 1931, and recently, in a thorough review of the subject of pulmonary vascular sclerosis, both primary and secondary, Brenner (2) found sixty-six case reports to which he added two of his own. For this reason another review of the subject at this time might seem unwarranted, but as a sequel to the clinical studies of Jennes (3) in the preceding paper in this Bulletin, the pathological findings of his second case, which were studied here, merit a report.

Clinical History: R. B., an unmarried white man, age 35, was admitted to the Pennsylvania Hospital on December 17, 1935, with a chief complaint of abdominal pain. The family history was irrelevant. He was born in Philadelphia and his infancy had been uneventful. As a child he had measles, mumps, and chickenpox. His mother stated that during early adolescence he was unable to exercise as strenuously as his acquaintances and tired readily. He had, however, been able to work irregularly as a ship's steward prior to 1929 and had travelled extensively in the Orient and in Central America. In 1925, at Tampico, Mexico, he had an attack of jaundice which lasted for a week but did not incapacitate him. In the same year he was told by a physician that he had heart disease. After that time he had some dyspnea on moderate exertion. During November 1934, because of a cold and pain in the chest, he attended the Medical Clinic of the Johns Hopkins Hospital. On his return to Philadelphia dyspnea on exertion became much more pronounced but he noticed no edema, cyanosis, or precordial pain. About a week before admission to this hospital he began to notice a feeling of heaviness, associated with intermittent dull aching pain, in the epigastric region. These symptoms apparently had no relation to meals and he had no nausea or vomiting. His appetite, previously excellent, became poor. Except for belching after meals, he had no previous gastro-intestinal symptoms. From the Johns Hopkins Hos-

pital it was learned that a diagnosis of emphysema, bronchitis, and hypotension (blood pressure 90/60) was made, and that a congenital abnormality of the heart or an aneurysm of the pulmonary artery was suspected.

On admission, the patient was well nourished and well developed. He was in no apparent distress. The blood pressure was 136/96. There was slight jaundice of the conjunctivae and sclerae. Slight pretibial edema was noted. The antero-posterior diameter of the chest was thought to be somewhat increased. Expansion of the lungs, however, was good and equal. The percussion note was slightly hyperresonant throughout. The breath sounds were physiological. An occasional medium crackling râle was heard at both bases posteriorly. Examination of the heart revealed a slight precordial bulge and marked precordial activity in the second, third, and fourth intercostal spaces to the left of the sternum. An inconstant systolic thrill and a definite diastolic thrill were palpated in the second left intercostal space. The heart was thought to be enlarged in all diameters. The rhythm was regular; the cardiac rate was 100. Over the base of the heart a loud harsh diastolic murmur and an inconstant rough systolic murmur were heard. These were loudest in the pulmonic area, but were occasionally transmitted to the apex. There was no Corrigan pulse. The abdomen was slightly distended and there was a definite fulness in the right upper quadrant. The liver was palpated as a firm, nodular, non-tender mass with rounded border extending to the level of the umbilicus. It did not pulsate. The remainder of the physical findings were essentially normal. At this time, in addition to a congenital heart lesion, the possibility of an echinococcus cyst of the liver was considered, but the onset of a rapidly progressive congestive failure prevented any surgical treatment. Orthopnea and marked edema of the dependent portions of the body, particularly of the lower extremities, ensued. Cyanosis, which was not observed on admission, now became marked. The jaundice deepened. The congestive failure grew worse and the patient died on January 17, 1936. The temperature was elevated slightly for several days after admission but was consistently subnormal for a week before death. Repeated examinations of the urine revealed only large amounts of bile and small quantities of albumin. The day after admission, a blood count showed the following: Hemoglobin 16 gm., Erythrocytes 4,980,000, Leucocytes 12,100 of which there were 70 per cent. polymorphonuclear neutrophiles, 1 per cent. eosinophiles, 27 per cent. lymphocytes and 2 per cent. monocytes. The blood Wassermann tests both here and at the Johns Hopkins Hospital were negative. One echinococcus complement fixation test was weakly positive; another was negative. The qualitative van den Bergh test was obscured by hemolysis; the quantitative color determination was equivalent to 4.5 mg. of bilirubin per 100 cc. of serum. The Takata Ara Test was positive. Total serum proteins were 6.90 gm., albumin was 3.7 gm., and globulin was 3.2 gm. per 100 cc. of blood. The albumin-globulin ratio was 1.19. An electrocardiogram showed regular sinus rhythm; the P.R. interval was 0.18 seconds; there was marked right axis deviation; T-2 and T-3 were inverted; Q.R.S. complex was slurred in lead 2; and the chest lead was normal. In roentgenograms of the chest and abdomen, the

heart was seen to be enlarged and there was an increased prominence of the pulmonary conus. In the region of the hila of both lungs numerous small round plaques, thought to be calcified, were seen. Some of these appeared to be arranged radially. The liver shadow was greatly enlarged and the right dome of the diaphragm was displaced upward. Two large irregular and numerous smaller calcified areas were seen in the liver substance. Other laboratory data were not important.

Autopsy (No. 5447): A partial autopsy was performed twelve hours after death. Permission was not obtained to examine the neck organs, brain, or spinal cord. *Anatomical Diagnosis:* Extensive arteriosclerosis and calcification of pulmonary arteries, coronary arteries, and aorta. Pulmonary arteriolosclerosis. Emphysema of lungs, slight. Fibrous strands of adhesions between lower lobes of both lungs and diaphragm. Fibrous adhesions at apex of right lung. Marked hypertrophy and dilatation of right auricle and ventricle. Diffuse scarring of right auricle and ventricle. Dilatation of pulmonic and tricuspid valves. Chronic passive congestion of lungs, liver, and spleen. Hydropericardium. Ascites. Peripheral edema. Clubbing of fingers, slight. Multiple liver-cell carcinoma of right lobe of liver with a metastasis to a small pulmonary artery. Extensive areas of calcification of liver. Jaundice. Hyperplasia of bone marrow, slight. Foci of extramedullary hematopoiesis in spleen.

Body: The body was that of a large well developed white man, measuring 172 cm. in length. The hair was sparse. A moderate jaundice of the skin, conjunctivae, and sclerae was present. There was no perforation of the nasal septum. The teeth were not of the Hutchinsonian type. A small amount of bloody froth was present in the mouth. The superficial veins of the neck were greatly distended. The thorax was thought to be of normal contour and did not appear to be significantly increased in its anteroposterior diameter. The abdomen was moderately distended. A scar of a former circumcision was present on the penis but no lesions which could be ascribed to syphilis. There was slight clubbing of the fingers and marked cyanosis of the nail beds of the hands and feet. There was marked edema of the legs, extending as high as the abdomen, but none of the upper extremities. No other abnormalities were noted.

On section, the thoracic and abdominal walls were edematous. There were about 200 cc. of clear, straw-colored fluid in the abdominal cavity, but no free fluid in either pleural cavity. A few fibrous strands of adhesions were present between both lower lobes of the lungs and the diaphragm, and a small dense band of adhesions existed between the right apex and the lateral thoracic wall. The liver, which extended 10 cm. below the right costal margin, weighed 2520 gm. and measured 28 by 31 by 11 cm. The left lobe of the liver was small and almost atrophic in appearance. The right lobe was greatly enlarged; its lower border was rounded and it contained numerous discrete and confluent nodules, varying in size from one to five centimeters in greatest diameter. These nodules were yellow and most of them were soft and necrotic. In the center of the largest necrotic mass were several large, irregular, calcified areas having the hardness of bone.

In these calcified areas were several small cysts, from 5 to 15 mm. in diameter, which contained small amounts of blood-tinged gelatinous material. Examination of the contents did not reveal the presence of echinococcus scolices. The left lobe and the small amount of relatively normal tissue remaining in the right lobe had a "nutmeg" appearance. There were no lesions of the gallbladder or lower biliary tract. The spleen weighed 420 grams and on section an increased redness of the pulp was seen. The remainder of the abdominal organs were grossly normal.

Heart, Lungs and Aorta: The heart was in its normal position. The pericardial sac contained about 125 cc. of clear, straw-colored fluid. There was no pulmonary embolus. The transverse diameter of the heart was 16 cm. The heart weighed 530 grams. Externally its right side was greatly enlarged and the anterior portion of the apex was formed entirely by the right ventricle. On section, the right auricle and ventricle were dilated and the ventricle was greatly hypertrophied. The endocardium was thickened and opaque. The left auricle and ventricle were neither dilated nor hypertrophied and the endocardium was thin and transparent. The right ventricle measured 13 mm. and the left measured 15 mm. in thickness. The orifice of the left coronary artery was of normal width, but the orifice of the right was appreciably narrowed by a calcified plaque in the intima of the sinus of Valsalva. The lumina of the coronary arteries were narrowed, but not occluded, by arteriosclerotic plaques containing flecks of calcium. There were no gross scars or other lesions in the myocardium. The valves were all thin and delicate. The circumferences of the valve rings were as follows: Tricuspid 11 cm.; pulmonic 8 cm.; mitral 6.5 cm.; aortic 5.5 cm. Slight dilatation of the pulmonic ring and slight incompetency of the pulmonic valve were thought to be present.

About one centimeter above the valve, the pulmonary artery measured 9 cm. in internal circumference. The intima was generally thickened and opaque with longitudinal wrinkles. In addition, there were numerous raised atheromatous patches from 2 mm. to 2 cm. in greatest diameter. On section, these plaques were composed of firm fibrous material without calcification. The underlying media appeared to be thinner than elsewhere. The right branch of the pulmonary artery measured 6 cm. and the left 5.5 cm. in internal circumference. On each side, the branches of the second order averaged about three centimeters in diameter and the tip of the little finger could readily be inserted into them to within one or two centimeters of the pleural surfaces. In branches of the first and second order, numerous elevated atheromatous plaques in the intima were present in even greater numbers than in the main pulmonary artery. The most striking feature, however, was the presence of numerous flat but elevated intimal plaques of calcification which corresponded to the areas of calcification seen in the roentgenograms. Most of these appeared to be on the verge of ulceration but evidently were still covered by endothelium. The largest of these lesions was 1.5 cm. in greatest diameter. Beneath these areas, the media was thinner than elsewhere. The smaller intrapulmonic branches of the artery were stiff and somewhat dilated. No saccular dilatations of the pulmonary artery or any of its branches were present.

The lungs were for the most part air-containing except at the bases where they were moist. Throughout the parenchyma there were patchy areas of slight rarefaction. No other lesions were seen.

The aorta was not dilated but had lost much of its elasticity in the abdominal portion. In the entire arch and thoracic portions, there were only scattered atheromatous flecks not more than 2 mm. in diameter. For several centimeters above the bifurcation, however, there was marked sclerosis with areas of calcification in the intima similar to those seen in the branches of the pulmonary artery.

Microscopic Lesions—Pulmonary Artery and Lungs: In sections of the main pulmonary artery the intima is uniformly thickened by proliferation of fibrous tissue. There are many nodular elevations of the intima often twice the width of the underlying media. These elevations are composed for the most part of dense fibrous tissue, in the deeper layers of which are occasional vacuolated cells, containing fat. Minute amounts of calcium are present and capillaries from the media invade these areas. Beneath the elevated sclerotic plaques, the media is somewhat thin and tiny scars are rarely present, but there is no cellular infiltration. The adventitia is slightly thickened and hyaline in appearance. Here too, there is no cellular infiltration. The same changes are present in the extrapulmonary and in the larger intrapulmonary branches but unlike the main pulmonary artery large areas of calcification are present in the intima, and the media beneath is thinner and more scarred. The adventitia composed of hyaline connective tissue is extremely thick. Nowhere is there any leucocytic infiltration in the media or adventitia. In the lungs, there is no calcification but there is marked sclerosis and adventitial thickening of most of the arteries. In numerous sections, the lumina of the arterioles smaller than one millimeter in diameter are greatly narrowed by marked intimal proliferation. Two or three are found which contain organized thrombi. One small artery is occluded by tumor cells, resembling abnormal liver cells, but nowhere else can any metastatic lesions be found. There are patchy areas of slight emphysema throughout all lobes of the lungs and in sections from both lower lobes there are moderate amounts of pulmonary edema and small collections of "heart failure" cells. The bronchi appear normal.

Heart and Aorta: In sections of the right ventricle there is marked hypertrophy of the muscle fibers and a moderate amount of diffuse scarring. There is also slight scarring and hypertrophy of the right auricle. Sections of the left auricle and ventricle, however, show no lesions. No evidence of recent or old myocardial infarction is seen. A section of the right coronary artery just behind the sinus of Valsalva shows the lumen to be narrowed but not occluded by a projecting plaque of calcification in the intima. There are occasional small atheromatous plaques in the arch and thoracic portion of the aorta, but there is marked calcification of the intima in the abdominal portion. The adventitial thickening seen in the pulmonary artery and its branches is absent in the aorta. There is no cellular infiltration.

Other Organs: In sections from the remaining organs, slight arteriosclerotic changes are rarely seen, and there is no arteriolosclerosis. The nodules in the liver,

large areas of which are necrotic, are composed of tumor cells of parenchymatous liver cell type. The cells show distinctly malignant characteristics. The large areas of calcification are surrounded by necrotic tissue. In the portions of liver not invaded by the tumor, there is extensive necrosis and atrophy of the central liver cells with hemorrhages about the central veins. An increased amount of fibrous tissue is present in the splenic pulp in which are moderate numbers of megakaryocytes and occasional minute areas of red blood cell formation. The splenic sinuses are distended with blood. Although there are considerable amounts of fatty tissue in the bone marrow of the ribs and vertebral bodies, there appears to be a slight hyperplasia of the erythroblastic elements and megakaryocytes are present in increased numbers. The remaining organs show no microscopic lesions.

DISCUSSION

The criteria by which a justifiable diagnosis of primary pulmonary arteriosclerosis may be established are not necessarily rigid. Strictly speaking, however, there should be no chronic pulmonary disease, no evidence of pre-existing heart disease, and no significant sclerosis elsewhere. On this basis, Brenner found that only sixteen of the sixty-eight cases in the literature were not open to question. Objections to such a diagnosis in this case are therefore apparent. Since there was no scarring or hypertrophy of the left ventricle, the sclerosis of the coronary arteries and right coronary orifice does not appear to have been responsible for a preceding left-sided failure to which the pulmonary sclerosis might have been secondary. By increasing the resistance to the flow of blood through the lungs, emphysema has been considered a cause of secondary pulmonary vascular sclerosis. The slight emphysema present in this case was not so marked as the clinical findings would indicate. That such a discrepancy may exist was observed by Hurtado, Fray, and McCann (4). These authors stated that an emphysematous appearance clinically may not represent emphysema pathologically. There is no evidence to suggest an obstructive origin of the emphysema in the present case. Christie and Meakins (5) suggested that the decreased distensibility of the alveoli in congestive heart failure resulted in patchy areas of emphysema. The chronic passive congestion of the lungs in this case, however, was so slight as to be considered a terminal event. Moreover, it is not clear that some cases of emphysema may not be the result rather than the cause of pulmonary arteriosclerosis. Although ne-

crosis does not occur, diminution in the blood supply of the capillaries may result from extensive narrowing of the small arteries and arterioles and in turn may cause atrophy and emphysema of the alveoli. It is believed that the emphysema in the present case had such an etiology. In other organs of the body there was little arteriosclerosis and no arteriolosclerosis. There is also no reason for believing that the sclerosis and calcification of the abdominal aorta had any influence on the development of the pulmonary vascular sclerosis. It seems more likely that the sclerosis of the aorta, coronary arteries, and pulmonary arteries occurred simultaneously. This case, therefore, is thought to be one of primary pulmonary arteriosclerosis.

Of additional interest in this case is the calcification of the pulmonary arteries. According to Brenner, such lesions are rare.

The pulmonic ring and the pulmonary arteries were thought to be dilated. Clarke, Coombs, Hadfield, and Todd (6) stated, however, that the normal circumference of the pulmonic ring is between 8.5 and 9 cm. Among fifteen normal adults Brenner found that the circumference of the pulmonary artery varied between 5.8 and 9.3 cm. The measurements of 8 cm. for the pulmonic ring and 9 cm. for the pulmonary artery in the present case are apparently within the upper limits of normal.

Rosenthal (7) believed that pulmonary vascular sclerosis represents a definite clinical and pathological entity, but MacCallum pointed out that the etiology of primary pulmonary arteriosclerosis is obscure. Of those cases, furthermore, which appear to be primary, there is considerable variation both in the type of arterial lesions present and in the ages of the patients. In MacCallum's case the lesions were predominantly arteriolar. The cases of Tchistovitch (8), Frothingham (9), and Goedel (10) showed extensive thrombosis, with organization, of the smaller pulmonary arteries. The oldest patient, aged 74 years, appears to be that of Schütte (11) and the youngest, aged 6 months, that of Bryant and White (12). Cyanosis had been present since three weeks of age in Darley and Doan's case (13), but the patient survived until the age of twenty.

Since there are probably several etiological factors and since there is so little consistency in the age incidence, it would seem preferable to consider primary pulmonary arteriosclerosis as a syndrome.

SUMMARY AND CONCLUSIONS

1. A case of primary pulmonary arteriosclerosis with calcification of the pulmonary arteries is presented and the evidence in favor of such a diagnosis is discussed.

2. It is suggested that some cases of emphysema may be the result rather than the cause of pulmonary vascular sclerosis.

3. Primary pulmonary arteriosclerosis is not a clinical and pathological entity but a syndrome.

REFERENCES

(1) MacCallum, W. G.: *Bull. Johns Hopkins Hosp.*, 1931, **49**, 37.
(2) Brenner, O.: *Arch. Int. Med.*, 1935, **56**, 211; 457; 724; 976; 1189.
(3) Jennes, S. W.: *Bull. Johns Hopkins Hosp.*, 1936, **51**, 133.
(4) Hurtado, A., Fray, W. W., and McCann, W. S.: *J. Clin. Investigation*, 1933, **12**, 833.
(5) Christie, R. V., and Meakins, J. C.: *ibid*, 1934, **13**, 323.
(6) Clarke, R. C., Coombs, C. F., Hadfield, G., and Todd, A. T.: *Quart. J. Med.*, 1927, **21**, 51.
(7) Rosenthal, S. R.: *Arch. Path.*, 1930, **10**, 717.
(8) Tchistovitch, T.: *C. rend. de la Soc. de biol.*, 1923, **89**, 627.
(9) Frothingham, C.: *Am. J. Path.*, 1929, **5**, 11.
(10) Goedel, A.: *Virch. Arch. f. path. Anat.*, 1930, **277**, 507.
(11) Schütte, H.: *Centralblatt f. allg. Path. u. path. Anat.*, 1914, **25**, 483.
(12) Bryant, J. H., and White, W. W.: *Guy's Hosp. Reports*, 1901, **55**, 17.
(13) Darley, W., and Doan, C. A.: *Am. J. Med. Sc.*, 1936, **191**, 633.

PLATE 1

Fig. 1. The heart, pulmonary arteries, and lungs. The right ventricle is hypertrophied and dilated. The pulmonary artery and its larger branches appear to be dilated.

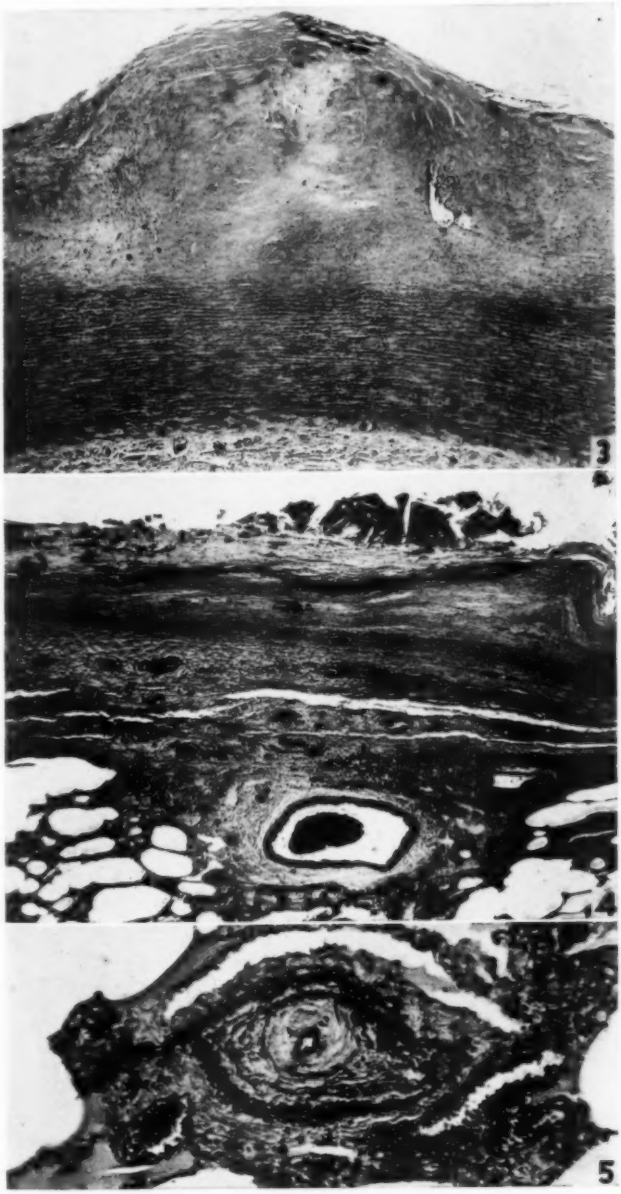
Fig. 2. A magnified segment of the left main branch of the pulmonary artery. Intimal wrinkling and plaques of calcification are shown.

PLATE 2

FIG. 3. A section of the main pulmonary artery showing a thick fibrous intimal plaque in which there is a large blood channel. The media is relatively normal.

FIG. 4. A decalcified section of a large intrapulmonic branch of the pulmonary artery. Areas of calcification are present in the intima. The media is thin. The adventitia is thickened. Adjacent to the adventitia is one of the few normal small arteries seen in the lungs.

FIG. 5. A pulmonary arteriole of about one millimeter in diameter (Verhoeff's Elastica Stain). The media is intact but there is marked proliferation of the intima.


3
4
5

SEVERE MYOCARDITIS OF UNKNOWN ETIOLOGY

HELEN B. TAUSSIG AND ELLA H. OPPENHEIMER

From the Harriet Lane Home of the Johns Hopkins Hospital and the Departments of Pediatrics and Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland

Submitted for publication July 15, 1936

The majority of cases of acute myocarditis occur during the course of a septicemia. In most instances the causative organism can be recovered from the blood stream or, at least, can be demonstrated at autopsy by a bacterial stain. The three great exceptions are acute rheumatic fever, tuberculosis and syphilis. In rheumatic fever, both the clinical and the pathological findings are usually so characteristic that the nature of the disease can generally be recognized with certainty.

When tuberculosis attacks the myocardium, it is either in the form of a localized tuberculous focus which may go on to caseation and even to rupture, or else it is part of a generalized miliary tuberculosis. Although the nature of the myocarditis may be difficult to recognize clinically, at autopsy it is usually found to be associated with a widespread tuberculous infection.

Syphilis deserves special consideration as an etiological factor. Although it is a disease which comparatively rarely affects the myocardium, it is now generally accepted that syphilis can cause a true myocarditis. The usual pathological picture is that seen late in the disease, as the majority of patients die in the tertiary stage. In these cases there is an extensive, diffuse, fine scarring throughout the myocardium with occasional areas of perivascular round-cell infiltration. It is in these small inflammatory areas that Warthin (1, 2) has succeeded in demonstrating spirochetes.

The problem remains whether these scars are produced by a long continued, low grade inflammation extending over a period of years, or whether the lesion is the final result of a previous acute myocarditis which, at the time, was asymptomatic. There is reason to believe that at least in some cases the latter may be the correct explanation.

A few cases of acute syphilitic myocarditis have been reported in patients who died early in the course of the disease.

Stoeckenius (3) in 1912 reported four cases of acute myocarditis occurring in patients with arsenical dermatitis who died in the secondary stage of syphilis. One died as a result of suicide; the other three died while suffering from a severe arsenical dermatitis. At autopsy all four cases showed extensive round-cell infiltration in the heart, kidneys and lungs. In none of these cases had the myocarditis been suspected during life. Inasmuch as all four patients were known to be suffering from secondary syphilis, the author thought that even though spirochetes could not be demonstrated, the inflammatory reaction was, in all probability, due to syphilis. Brooks (4, 5), in 1913, reported two cases of syphilitic myocarditis occurring during the secondary stage of syphilis. The first was in a woman of 38 years with a well developed secondary rash who died suddenly, and at autopsy was found to have an acute syphilitic myocarditis; the second was in a man who died of a ruptured aneurysm before the secondary manifestations had fully appeared, and in this case also autopsy revealed an extensive acute myocarditis. Boyd (6) has reported a case of acute myocarditis in a patient, 60 years of age, in which spirochetes were demonstrated in the myocardium. Wile, Wieder, and Warthin (7, 8) reported a case of severe syphilitic myocarditis in a young man with malignant syphilis in whom the disease ran a rapidly fatal course in spite of all therapy. Recently, Hamman and Rich (9) have reported a case of myocarditis which they believed was syphilitic in origin even though spirochetes could not be demonstrated at autopsy.

In addition to these specific infections, there is another group of cases in which an acute interstitial myocarditis of unknown etiology is found at autopsy. Essentially similar cases of myocarditis have been reported under various names—for example, "acute interstitial myocarditis" (10, 11), "Fiedler's myocarditis" (12), or "isolated myocarditis" (13, 14, 15). The outstanding feature in these cases is that the myocardium is the seat of an inflammatory reaction, while the other organs of the body are spared. Whether the process is due to a toxic agent or to some obscure organism is not known. In a few cases, the myocarditis has occurred in association with a severe arsenical dermatitis (16, 17). No bacteria have ever been cultured or stained,

and no single etiological agent has been recognized. Ordinarily, the inflammation is found only in the myocardium. There is, however, no obvious reason for this to be an essential characteristic of the disease.

In the following case, the child suffered from both tuberculosis and syphilis. Whether the myocarditis was due to one of these diseases or was due to some unknown etiological agent, as in the acute interstitial myocarditis, could not be conclusively determined. Nevertheless, the case presents a most unusual picture and is therefore reported in detail.

CASE REPORT

D. H., colored girl, age 6 years. (H. L. H. No. 45821.)

Family History: Non-contributory. *Past History:* Birth normal. There was no history of syphilis. The maternal blood Wassermann was negative.

First Admission to the Harriet Lane Home was at the age of 1 year because of an acute gastrointestinal disturbance. The only significant positive finding was enlargement of the spleen. The tuberculin test with 0.1 mg. of tuberculin was negative. The X-ray of the extremities showed no evidence of congenital syphilis. The blood Wassermann was negative. A fresh blood preparation showed no sickling of the red blood cells.

The child had had pertussis and a severe streptococcal tonsillitis in infancy. At 2 years of age she had developed an acute gonococcal vaginitis. At the age of 5 she was readmitted to the hospital with an acute cervical adenitis and a severe anemia.

Second Admission to Harriet Lane Home, October, 1930, to June, 1931, at the age of 5 years.

Physical examination showed the following positive findings: There was marked undernutrition. The sclerae had a slight icteric tint. The mucous membranes were pale. The heart showed slight enlargement and a soft functional systolic murmur. The lungs were clear. The liver and spleen were enlarged—both extended three fingerbreadths below the costal margin.

Laboratory findings: The red blood cell count was 1,700,000. Hemoglobin 35 per cent (Sahli). The fresh blood preparation showed immediate sickling of the red blood cells. The leucocyte count was 33,800. The blood smear showed many immature white blood cells. The urine examination was negative except for the presence of urobilin. The blood Wassermann was negative. The skin reaction to 0.1 mg. of tuberculin was strongly positive. An X-ray of the chest showed enlargement of the mediastinal glands. The electrocardiogram was normal.

Clinical diagnosis: Undernutrition, sickle-cell anemia, and tuberculosis of the mediastinal glands.

Treatment in the Hospital: Owing to the severe manifestations of the sickle-

cell anemia, the child was given three transfusions—the first on November 4, the second on December 8, and the third on January 22.

In an effort to prevent the exacerbations of the sickle-cell anemia, the child was treated with intramuscular injections of human serum (18). She received three injections[1] while in the hospital, and in June was sent home to continue treatments in the dispensary.

Between July 6 and October 10 (inclusive) the patient returned regularly every two weeks. On each visit she was given 10 c.c. of her mother's blood intramuscularly, thus receiving eight injections in all. On October 29 her mother was in jail for drunkenness, and hence no blood was given.

On November 2 the patient developed signs of secondary syphilis—fever, general glandular enlargement, and a widespread papular rash. The blood Wasser-

TABLE I

Treatments given in syphilis clinic

DATE	TEMPERATURE	WEIGHT	AMOUNT OF SULPHARSPHENAMINE GIVEN INTRAMUSCULARLY	GENERAL CONDITION
		pounds	*grams*	
11/11/31	100°	33	0.14	Good
11/18/31	99	32.5	0.28	Excellent
11/25/31	98.6	32.2	0.28	Excellent
12/ 2/31	98.6	33.5	0.28	Excellent
12/ 8/31	Patient failed to return for treatment			
12/15/31	98°	35	0.28	Excellent
12/23/31	98	34	0.28	Excellent
12/30/31	98	34.8	0.32	Excellent
1/ 6/32	Patient failed to return for treatment			
1/13/32	98.6°	35.5	0.32	Excellent
1/20/32	99.6	34.4	0.32	Breaking out of five days' duration

mann was positive. The mother had been used as donor for all transfusions and for all subsequent intramuscular injections of whole blood and of serum. Her Wassermann was negative at the beginning, but it was not retested prior to each subsequent treatment. When the child's Wassermann was found to have become positive, both the mother's Wassermann as well as the child's was immediately retested. Both were positive. They were promptly referred to the adult and pediatric syphilis clinics.

From November 11 to December 30, the patient returned regularly for antisyphilitic treatment, except on December 8 and on January 6 when she failed to report (Table 1). During this period, she became afebrile, gained in weight,

[1] Dates: March 9—50 c.c. intramuscularly. May 26 and June 2—15 c.c. intramuscularly.

and seemed symptomatically better. On January 15 she developed a skin eruption which was thought to be either lichen scrofulosorum or lichen syphiliticum. Five days later, she was admitted to the Harriet Lane Home for study of this condition.

Third Admission to Harriet Lane Home, January 20 to January 29, 1932, at the age of 6 years.

Physical Examination: Temperature, 37.4°C. Pulse, 100 per minute. Respirations, 26 per minute. Blood pressure, 100/50.

The patient was not acutely ill. Indeed, she was bright, lively, and active; her only complaint was a mild pruritus. The skin was covered with myriads of tiny discrete papules which were slightly elevated and brownish with sunken umbilicated centers (as illustrated in Figure 1). According to the dermatological report, the eruption could be either tuberculous or syphilitic. There was pronounced general glandular enlargement. An X-ray taken January 22, 1932 showed that the heart was definitely enlarged. The character of the murmur was essentially the same as upon previous examinations. The lungs were clear. The liver edge was smooth and firm and readily palpable three fingerbreadths below the costal margin. The spleen was enormously enlarged; it extended almost to the level of the anterior superior spine. The genitalia appeared normal. There were some papules on the labia majora but no labial condylomata. The extremities were essentially negative. The reflexes were normal.

The *clinical impression* on admission was: Sickle cell anemia, lichen scrofulosorum, acquired syphilis with healed secondary lesions, and tuberculosis of the mediastinal glands.

Laboratory Examination: The red blood cell count was 3,335,000. Hemoglobin 50 per cent (Sahli). The leucocyte count was 9,500 with 77 per cent polymorphonuclear cells, 20 per cent lymphocytes, and 3 per cent myelocytes. There was a slight amount of sickling in ten minutes; 50 to 70 per cent of the cells showed sickling in twelve hours. The urine was negative.

Course in the Hospital: Immediately after admission the child developed a hectic temperature. On the third day, she complained of severe pain in the spine. Two days later, she became markedly dyspneic, complained of generalized pain, and developed acute myocardial failure. The hemoglobin dropped to 25 per cent. The electrocardiogram showed a sinus tachycardia and curves of low voltage (resistance, 1800 ohms). Four days later, in spite of all therapy, she died of cardiac failure.

At the time of death, the blood Wassermann was negative. The blood cultures taken on January 25 and January 30 were sterile.

The final clinical impression was: Acute myocardial failure, sickle-cell anemia, acquired syphilis, tuberculosis of the mediastinal glands, and lichen scrofulosorum or lichen syphiliticum.

Autopsy, No. 12399, (Dr. Brawner) showed the following significant gross lesions:

External Appearance: There was marked emaciation. The skin showed a gen-

eralized eruption which was dry, slightly raised, and of a fine granular character: it was most prominent in the suprapubic and cervical regions. These lesions represented a dried eruption which, in most places, was discrete although in some areas it had become confluent.

FIG. 1. PHOTOGRAPH OF THE SKIN SHOWING THE DISTRIBUTION AND CHARACTER OF THE LICHEN SYPHILITICUM ON THE BACK

Pericardial, pleural, and abdominal cavities: There were 250 c.c. of turbid yellow fluid in the pericardium. The pleural cavities contained only a few cubic centimeters of clear fluid. The abdominal cavity contained 150 c.c. of clear, straw-colored fluid.

The *mediastinal lymph nodes* were enlarged and yellowish in appearance. On section, several small caseous areas were seen. There were calcified areas in the lymph node at the bifurcation of the trachea.

The *heart* was markedly enlarged; it weighed 260 gms. The pericardium was roughened by a fresh fibrinous exudate near the apex. The myocardium showed an extraordinary pallor which appeared to be due to a large number of minute flecks. Some of these areas were as large as 7 mm. in width. No gross scarring was evident. Both ventricles were markedly dilated. In the left ventricle beneath the site of the fibrinous exudate, there was a small, rather firm, discrete, yellowish nodule which resembled a tubercle. Attached to the endocardium at this site, there were small mural thrombi. The valves were all thin and delicate.

The *lungs* showed a few fibrous adhesions about the right lower lobe but no definite pneumonia. Several small firm nodules were felt in the periphery of the left lung. A caseous nodule, thought to be a primary tuberculous focus, was found in the right lower lobe. The corresponding hilum node in its central portion contained a caseous area.

The *liver* was enlarged; it weighed 850 gms. and extended 6 cm. below the costal margin. There were scattered little gray flecks visible through the capsule. The *spleen* was enormous, weighing 175 gms. It felt firm and rubbery. There were some old adhesions about the capsule. The pulp was of a uniform brownish-red color. Malpighian bodies were not visible. There were numerous scattered orange-yellow flecks resembling pigment; in some places, these appeared to follow the trabeculae. The *gastrointestinal tract* was normal except that the Peyer's patches and solitary follicles in the ileum were rather prominent. The *pancreas* and *adrenals* showed no gross lesions. The *kidneys* were of normal size. On section there were numerous blotchy gray areas, rather indistinctly outlined. Some of these areas were as wide as 6 mm. The tissues surrounding them were hyperemic. In addition, there were scattered punctate hemorrhages in the lining of the pelves. The *bladder* and *reproductive organs* were normal in appearance. The *bone marrow* of the rib, femur, and vertebra was deep red in color.

The heart's blood, pericardial fluid, and peritoneal fluid showed no organisms on direct smear and were sterile on culture.

Summary of Microscopic Lesions: A section of *skin* taken to show the lesion in the suprapubic region showed a normal intact, squamous epithelium with a normal amount of melanotic pigment. In the tissue just beneath the Malpighian layer, there was a very marked perivascular round-cell infiltration. There was no actual ulceration of the skin, although in some places the round-cell infiltration approached very close to the epithelium.

The *heart* showed the most extraordinary lesions. Throughout the entire myocardium of the ventricle and auricle, there was a dense, diffuse infiltration of mononuclear wandering cells. The round-cell infiltration was so diffuse throughout the myocardium that most of the muscle fibers had been destroyed. (See Figure 2.) In some areas the process was so extensive that there was definite necrosis. In such places, there were clusters of epithelioid cells and even giant

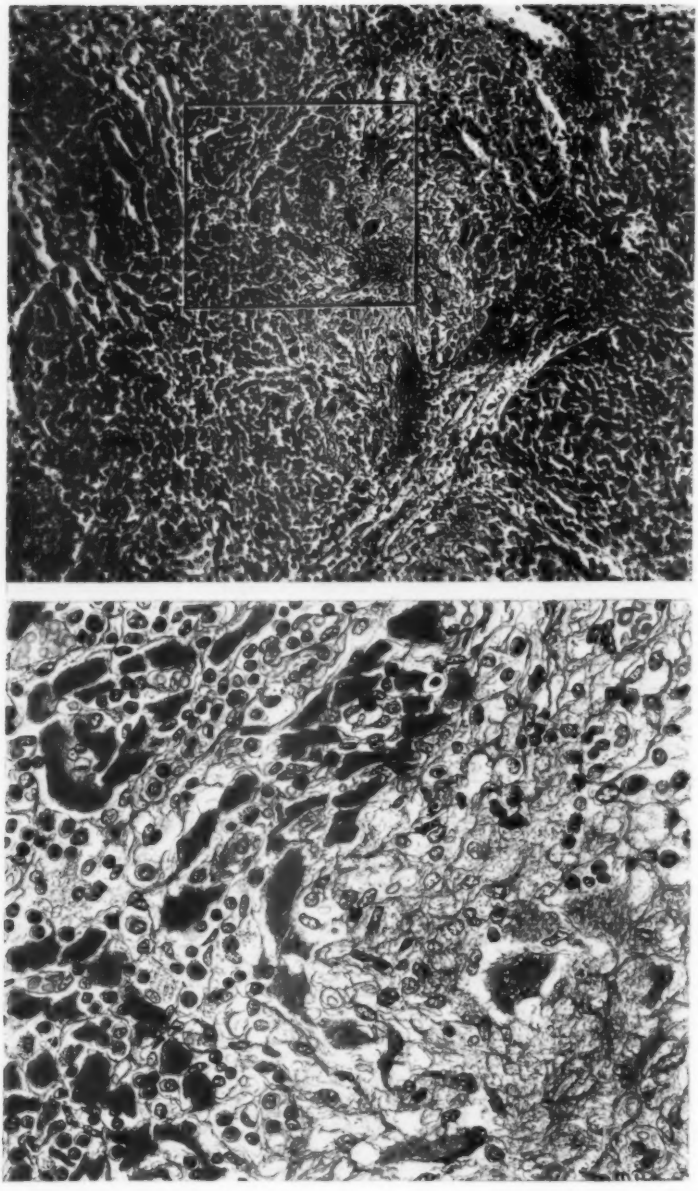


FIG. 2. ABOVE. MAGNIFICATION × 115 OF THE HEART SHOWING THE DIFFUSE MYOCARDIAL INFILTRATION AND A TUBERCLE-LIKE LESION CONTAINING A GIANT CELL

FIG. 3. BELOW. DRAWING AT A HIGHER MAGNIFICATION OF THE AREA MARKED IN FIGURE 2

cells about the central area of necrosis (as illustrated in the high-power drawing shown in Figure 3). The muscle fibers which did remain appeared somewhat enlarged and hypertrophied. The caseous nodule seen in the gross proved on section to show the same type of necrosis and proliferative inflammation as was found throughout the myocardium. There was a mural thrombus in the wall of the ventricle, and another was found in the wall of the right auricle. The pericardium and epicardium were thickened and also showed round-cell infiltration. The valves were unaffected. Fat stains of the heart muscle showed the presence of very little fat. Stains for ordinary bacteria and for tubercle bacilli revealed no organisms even after long searching. Many attempts were made to stain spirochetes,[2] but none could be demonstrated in the myocardium.

Scattered throughout both *lungs*, were small tubercle-like masses. These consisted of collections of epithelioid-like cells, a few giant cells, and in some places the centers of these lesions were slightly necrotic. No tubercle bacilli could be found. The alveoli showed no exudate and no evidence of pneumonia.

In the bronchial *lymph nodes*, there were necrotic lesions very similar to those seen in tuberculosis. Giant cells were present, but acid-fast stains revealed no organisms. Similar lesions were also seen in the lymph nodes taken from the retroperitoneal region. Section of the peritracheal node showed an old caseous area with powdery calcification. About this there was a connective-tissue capsule infiltrated with lymphocytes. The remainder of the node showed almost confluent areas similar to the previously described tubercle-like lesion. No tubercle bacilli could be stained either in the fresh lesions or in the very old calcified and obviously tuberculous area.

The *liver* showed a great accumulation of blood about the central veins, and the liver cells were definitely necrotic in the central zone of each lobule (as shown in Figure 4). Focal necroses were seen throughout the liver substance, and there were also several large concentric lesions resembling those described in the myocardium, the lungs, and the lymph nodes. On the whole, these large lesions showed less preservation and contained larger areas of necrosis than those found in the other organs. In some of the periportal areas, there were foci of round-cell infiltration.

---

[2] The following methods were used for the staining of spirochetes: (1) Levaditi stain; (2) Fontana stain; (3) Krajian modification of Dieterle's method. (*Am. J. Syph.*, **17**, 127–129, 1933).

Dr. Carl V. Weller also kindly undertook to examine the material for spirochetes even though we were unable to furnish him with freshly fixed formalin material, thereby rendering the staining of spirochetes very difficult. He did, however, demonstrate a spirochete in the wall of the bladder.

No dark field examination was made of the fresh material, as syphilis was not suspected at autopsy. However, frozen sections and scrapings of formalin-fixed myocardium were examined by dark-field illumination, and no spirochetes were seen.

The blood sinuses of the *spleen* were dilated and very congested. Malpighian bodies were rather small and, for the most part, were surrounded by lakes of blood, *i.e.*, showing the characteristic picture of sickle-cell anemia. None of the characteristic tubercle-like lesions were seen in any of the sections taken from the spleen.

Both kidneys showed numerous areas of interstitial round-cell infiltration. The glomeruli and tubules outside of these areas were essentially normal, and so were the kidney pelves.

The *pancreas*, *adrenals*, and *intestines* were normal.

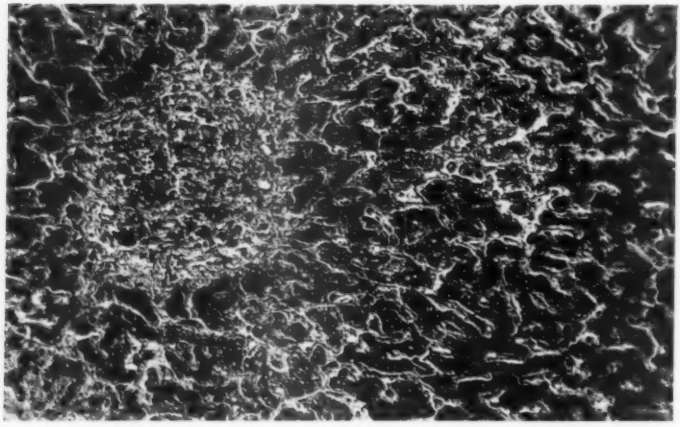


FIG. 4. MAGNIFICATION × 115 OF THE LIVER SHOWING AN AREA OF NECROSIS DUE TO CHRONIC PASSIVE CONGESTION, AND A NONSPECIFIC AREA OF FOCAL NECROSIS

The *bone marrow* was definitely hyperplastic. The majority of the cells in the femur marrow appeared to be of the erythrogenic series.

Anatomical Diagnosis: Sickle-cell anemia. Typical splenic changes and hyperplasia of the bone marrow. Tubercle-like coalescent areas with necrosis and giant cells throughout the myocardium, liver, lungs, and lymph nodes (syphilitic? —no spirochetes found). Generalized enlargement of the lymph nodes. Cardiac hypertrophy and dilatation. Mural thrombi, left ventricle. Sero-fibrinous pericarditis. Chronic passive congestion of the liver. Ascites. Enlarged Peyer's patches. Calcified bronchial lymph node.

DISCUSSION

To sum up: The case is one of a child in whom there was clinical and laboratory evidence of tuberculosis of the mediastinal glands and

sickle-cell anemia. She was treated with repeated blood transfusions and intramuscular injections of whole blood and thereby acquired a syphilitic infection. Immediately after the development of secondary syphilis, she was given antisyphilitic therapy. At first she appeared to respond well to treatment, but two months later, *i.e.*, after the eighth treatment, she developed a skin eruption and a week later died of cardiac failure. The postmortem Wassermann was negative. The autopsy revealed an extensive, diffuse myocarditis with caseation, tubercle formation, and giant cells. Similar, though less extensive, lesions were found in the lungs, liver, and lymph nodes. All cultures were sterile. All efforts to stain organisms failed. This does not exclude the possibility of syphilis because it is notoriously difficult to stain spirochetes, especially in cases which have received arsphenamine treatment. Although the etiology remains obscure, there are several factors which deserve consideration—namely, sickle-cell anemia, tuberculosis, and syphilis.

As regards sickle-cell anemia, Rich (19) has shown that the fundamental lesion is that of a congenital malformation of the splenic sinuses surrounding the Malpighian bodies. Although the heart is frequently enlarged, nothing in the nature of an acute myocarditis, such as occurred in this case, has ever been described. Furthermore, there are no reports in the literature even suggesting that either tuberculosis or syphilis is liable to run a more severe course in patients with sickle-cell anemia than in those free from this blood dyscrasia. Therefore, it does not seem probable that the sickle-cell anemia, *per se*, played a fundamental rôle in this pathological picture.

The changes in the myocardium were clearly those of a proliferative inflammatory reaction with giant cells and tubercle formation and caseation—a type of lesion seen both in tuberculosis and in syphilis. The child was known to have suffered from both of these infections. For this reason the problem presents itself whether either of the diseases could have been responsible for this remarkable and atypical picture.

As regards tuberculosis, although the characteristic proliferative lesion in the myocardium, abdominal and thoracic lymph nodes, and lungs closely resembled the specific lesion of tuberculosis, it differed in several important particulars. The distribution of lesions clearly indi-

cated a vascular dissemination of the causative agent. In tuberculosis, the spleen and liver are particularly apt to filter out blood-borne tubercle bacilli. In this case, however, there was only an occasional lesion found in the liver and not a single lesion in the spleen. Moreover, the myocardium was so completely altered by the specific lesion that a fatal myocarditis had resulted; whereas, in tuberculosis, even with a miliary distribution of the bacilli, at most only an occasional tubercle is found in the heart.

Furthermore, it is a relatively simple procedure to stain tubercle bacilli in fresh tubercles. Therefore, if the process had been tuberculous, tubercle bacilli should have been demonstrable in the myocardium. However, after painstaking search, none were found. The failure to demonstrate tubercle bacilli in the myocardium is not invalidated by the failure to find acid-fast bacilli in the calcified area in the tracheal lymph node, for such old lesions rarely reveal any organisms.

Because of the peculiar distribution of the lesion and of the failure to stain tubercle bacilli, tuberculosis seems to be definitely excluded as the etiological agent.

As regards syphilis, the picture is certainly not the usual one, but syphilis is notorious for its protean manifestations. In this instance, syphilis must be seriously considered, because the child was known to have acquired a syphilitic infection. There was, however, a brief period of apparent clinical improvement before the abrupt terminal illness. This raises the question whether death was due to some untoward result of the antisyphilitic treatment rather than to the direct ravages of the disease.

Let us review the various factors involved in the syphilitic infection. The child suffered from a syphilitic infection acquired either as the result of a blood transfusion, or, more probably, as the result of the intramuscular injections of whole blood. The question naturally arises whether syphilis as a result of blood transfusion is liable to run a more severe course than that acquired in the usual way. A review of the literature (20–44) gives no indication that such is the case.

Inasmuch as two to three months is the usual time interval between the injection of syphilitic blood and the development of secondary syphilis, the presumptive evidence in this instance is that the syphilitic

infection resulted from the intramuscular injections of whole blood rather than from transfusion.[3] Although the quantity of blood injected intramuscularly was small (10 c.c. each time) it is entirely possible that spirochetes were injected on more than one occasion. This was in all probability not a significant factor, inasmuch as superinfection in syphilis is not known to cause an unduly severe reaction (45).

The other question to be considered is whether the overwhelming infection was due to some untoward result of treatment such as a Herxheimer reaction or something similar to a neurorecidive. All that we can say is that no pathological lesion of this kind has ever been reported (46). Indeed, if the process was syphilitic, it must be a case of early malignant syphilis in a patient entirely resistant to treatment (47). Such cases have been reported with healing secondary lesions and a negative Wassermann reaction. However, in this case, no spirochetes were demonstrated in the myocardium.

SUMMARY

The case is one of a child who developed a syphilitic infection following the intramuscular injections of whole blood. At first she responded well to treatment, and then suddenly two months later developed a skin eruption and died of cardiac failure. Autopsy revealed an extensive, diffuse myocarditis composed of round-cell infiltration and tubercle-like proliferative lesions. There were similar lesions in the lungs, liver, and lymph nodes.

Even though the clinical events preceding death make syphilis appear to be the most probable cause of the disease, and the pathological findings are also consistent with a severe syphilitic infection, we were unable to establish the diagnosis. Consequently, although we believe the disease may have been syphilitic in origin, it is entirely possible that it was caused by some other unidentified infectious agent. The case, however, is so remarkable and the lesions so striking that we desire to put it on record in the hope that through the further reports of other similar cases the nature of this disease may ultimately be recognized with certainty.

[3] The last transfusion was given on January 22, 1931, and the syphilitic eruption did not appear until November 2, 1931.

REFERENCES

(1) WARTHIN, A. S.: Primary Tissue Lesion in the Heart Produced by Spirocheta Pallida. *Am. J. Med. Sc.*, **147:** 667–672, 1914.

(2) *Idem:* Sudden Death Due to Exacerbations of Latent Syphilitic Myocarditis. *Am. Heart J.*, **1:** 1–24, 1925.

(3) STOECKENIUS, W.: Beobachtungen an Todesfällen bei Frischer Syphilis. *Ziegler's Beitr. zu path. Anat. u. allgemeinen Path.*, **68:** 185–212, 1921.

(4) BROOKS, H.: The Heart in Syphilis. *Am. J. Med. Sc.*, **146:** 513–531, 1913.

(5) BROOKS, H., AND CARROL, J. H.: The Symptoms and Diagnosis of Involvement of the Heart in Syphilis (based on a study of 200 cases). *N. Y. State J. Med.*, **13:** 328–335, 1913.

(6) BOYD, W.: Acute Myocardial Syphilis. *Arch. Path. and Lab. Med.*, **2:** 340–342, 1926.

(7) WILE, U. J., WIEDER, L., AND WARTHIN, A. S.: Malignant Syphilis. *Am. J. Syph.*, **14:** 1–34, 1930.

(8) WARTHIN, A. S.: Extensive Diffuse Syphilitic Myocarditis Associated with Malignant Syphilis. *Ibid.*, **14:** 35–42, 1930.

(9) HAMMAN, L., AND RICH, A. R.: Clinical Pathological Conference: A Case of Syphilitic Myocarditis. *Internat. Cl.*, **4:** *series 44*, 221–254, 1934.

(10) BAILEY, F. R., AND ANDERSEN, D. H.: Acute Interstitial Myocarditis. *Am. Heart J.*, **6:** 338–348, 1930–31.

(11) LEGRAND, R., AND NAYRAC, P.: Un Cas de Myocardite interstitielle, diffuse isolée. avec rupture du coeur. Echo méd. du Nord., **32:** 590–601, 1935.

(12) FIEDLER, A.: Über akute interstizielle Myokarditis. *Festschrift zur Feier des fünfzigjährigen Bestehens des Stadtkrankenhauses zu Dresden-Friedrichstadt, Theil II*, 1–20, 1899.

(13) DE LA CHAPELLE, C. E., AND GRAEF, I.: Acute Isolated Myocarditis, with Report of a Case. *Arch. Int. Med.*, **47:** 942–956, 1931.

(14) SCOTT, R. W., AND SAPHIR, O.: Acute Isolated Myocarditis. *Am. Heart J.*, **5:** 128–141, 1929.

(15) MILLER, J.: Granulomatous Myocarditis. *Can. Med. A. J.*, **29:** 134–137, 1935.

(16) MAXWELL, E. S., AND BARRET, C. C.: Acute Interstitial Myocarditis: Report of a Case Following Severe Dermatitis Due to Sulphur Ointment. *Arch. Dermat. and Syph.*, **29:** 382–386, 1934.

(17) NELSON, R. L.: Acute Diffuse Myocarditis Following Exfoliative Dermatitis. *Am. Heart J.*, **9:** 813–816, 1934.

(18) JOSEPHS, H. W.: Influence of Plasma and Plasma Extracts on the Course of Hemolytic Anemia. *Am. J. Dis. Child.*, **48:** 932, 1934.

(19) RICH, A. R.: The Splenic Lesion in Sickle Cell Anemia. *Bull. Johns Hopkins Hosp.*, **43:** 398–399, 1928.

(20) AUBERTIN, C., AND FLEURY, J.: Syphilis après transfusion sanguine. *Bull. et mém. Soc. méd. d. hôp. de Paris*, **54:** 69–75, 1930.

(21) BREM, W. V.: Discussion of paper by Giffen, H. Z., AND HAINES, S. F. on "A Review of a Group of Professional Donors." *J. A. M. A.*, **81:** 535, 1923.

(22) CARLES, T. J.: Secundarismo por transfusion sanguinea. *Ecos Espãnoles de Dermatologia y. Sifiliografia*, 7ª anne no. 65, 287, 1930–31.

(23) CARNOT, P., CAROLI, AND MAISON: Syphilis decapitée par transfusion sanguine. *Bull. et mém. Soc. méd. d. hôp. de Paris*, **50:** 411–415, 1934.

(24) CONSTANTINESCOU, E., AND VATAMANU, N.: Un cas de syphilis d'emblée par transfusion sanguine. *Ann. d. mal. vén.*, **24:** 161–165, 1929.

(25) CORDIVIOLA, L. A.: Sífilis por transfusión sanguinea. *Semana Med.*, **1:** 43–54, 1932.

(26) CUMMER, C. L.: Transfusion Syphilis. *Am. J. Med. Sc.*, **185:** 787–789, 1933.

(27) DUFOUR, H.: Transfusion sanguine et syphilis. *Bull. et mém. Soc. méd. d. hôp. de Paris*, **53:** 511–514, 1929.

(28) FELDMAN, V.: Contribution a l'étude de l'infection hematogène syphilitique. *Rev. Franç. de dermat. et de vénérol.*, **4:** 341–350, 1928.

(29) FIESSINGER, N.: La syphilis par transfusion sanguine. *Prophylaxie Antivénérienne*, **4:** 56–58, 1932.

(30) FORDYCE, J. A.: Some Problems in the Pathology of Syphilis. *Am. J. Med. Sc.*, **149:** 781–808, 1915.

(31) GOUGEROT, FIESSINGER, BRUNO, AND DALLY: Deux cas de syphilisation par transfusion pour rajeunissement. *Bull. Soc. Franç. de dermat. et de syph.*, **37:** 1276–1278, 1930.

(32) JONES, H. W., RATHMELL, T. K., AND WAGNER, G.: The Transmission of Syphilis by Blood Transfusion. *Am. J. Syph. and Neurol.*, **19:** 30–38, 1935.

(33) LEVENT, R.: Syphilis par transfusion sanguine. *Gaz. des Hôp.* **103:** 1720–1725, 1930.

(34) LEVY, I. I., AND GINSBURG, L.: Syphilis by Blood Transfusion. *Am. J. Syph.*, **11:** 447–449, 1927.

(35) MOORE, J. E.: The Modern Treatment of Syphilis. P. 456. (Published by Charles C. Thomas, 1933.)

(36) MORGAN, H. J.: Factors Conditioning the Transmission of Syphilis by Blood Transfusion. *Am. J. Med. Sc.*, **189:** 803–813, 1935.

(37) PERIN, L., AND LEFÈVRE, M.: Transmission de la syphilis par voie sanguine. Contamination syphilitique d'une fillette à la suite de transfusions sanguines d'origine maternelle. *Bull. Soç. Franç. de dermat. et de syph.*, **42:** 954–957, 1935.

(38) PINARD, M., AND ROBERT, P.: Métrorragies de la puberté, transfusion, syphilis consecutive. *Bull. et mém. Soc. méd. d. hôp. de Paris*, **48:** 214–217, 1932.

(39) POLAYES, S. H., AND LEDERER, M.: Transmission of Syphilis by Blood Transfusion. *Am. J. Syph.*, **15:** 72–80, 1931.

(40) POST, C. D., AND COONEY, G. C.: Accidental Transmission of Syphilis by Blood Transfusion. *J. A. M. A.*, **100:** 258–259, 1933.

(41) SCHULMANN, E.: La syphilis sans chancre. *Pract. méd. Franç.*, **8:** 393–404, 1929.

(42) SPILLMAN, L., AND MOREL: Un cas de syphilis veineuse d'emblée. *Bull. Soc. Franç. de dermat. et de syph.*, **33:** 452–453, 1926.

(43) TZANCK, A., AND WERTH: Syphilis et transfusion sanguine (Absence de contamination en cas de donneur syphilitique latent). *Bull. et mém. Soc. méd. d. hôp. de Paris*, **54:** 132–133, 1930.

(44) WILLIAMSON, G. R., AND STRONG, R. A.: Congenital Syphilis from Blood Transfusion to Mother During Pregnancy. *Am. J. Syph.*, **17:** 484–491, 1933.

(45) CHESNEY, A. M.: Immunity in Syphilis. *Medicine*, **5:** 463–547, 1926.

(46) MOORE, J. E.: The Modern Treatment of Syphilis (Chapter 7, 14, and 16) (Published by Charles C. Thomas, 1933.)

(47) BEERMAN, H.: The Problem of Treatment of Resistant Syphilis. *Am. J. Syph.*, **20:** 165–206; 296–334, 1936.

THE MECHANISM OF A HITHERTO UNEXPLAINED FORM OF NATIVE IMMUNITY TO THE TYPE III PNEUMOCOCCUS

ARNOLD R. RICH AND CLARA M. McKEE

From the Department of Pathology of the Johns Hopkins Medical School, and the Department of Immunology of the Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland

Submitted for publication July 27, 1936

In most instances of native and acquired immunity there are demonstrable mechanisms which operate to limit the growth and spread of the bacteria and to destroy them. Ingestion and destruction of the bacteria by the phagocytes under the influence of native or acquired opsonin is a familiar and readily demonstrable protective process; the normal body temperature, or the composition of the body fluids, may be incompatible with the life or the proliferation of the bacteria; in a few instances specific lysins capable of bringing about the extracellular dissolution of the bacteria occur; antitoxins or native insusceptibility may shield the tissues from the injurious effects of toxins; and, as we were able to show several years ago, antibody plays an important rôle in preventing the spread of bacteria from the site at which they lodge (1) (2).

There are, however, certain instances of native and acquired immunity in which the mechanism responsible for the resistance is not yet clear. Among these instances tuberculosis occupies an important place. The phagocytes of both the non-immune and the immune animal will ingest the bacilli, but eventual intracellular destruction of the bacilli occurs to an incomparably greater degree in the immune animal. Furthermore, while extracellular death and lysis of the bacilli take place in the immune body in certain situations (*e.g.*, in caseous areas), the reason for this is not clear, for lysis of bacilli which have free access to the body fluids (*e.g.*, in the peritoneal cavity) has never been satisfactorily demonstrated.

Much has been written, for the most part in a rather vague manner,

concerning a hypothetical native or acquired "tissue immunity" to explain instances of resistance in which the mechanism is not clear, but no power of the tissues to bring about the destruction of bacteria independently of the activity of the phagocytes has ever been demonstrated. On the contrary, in a previous study (1) we have shown that even when animals are rendered highly resistant to virulent pneumococci by active immunization and are then deprived of their phagocytes, pneumococci will grow freely in their tissues and produce overwhelming and fatal infection, even though the plasma remains rich in antibody and potent in protecting animals possessing leucocytes.

In the present need for a better understanding of the precise nature of the immunity process in the infections in which the known mechanisms of resistance appear inadequate to account for the observed resistance, it is highly desirable to examine carefully any suggestive possibility of the existence of immunity mechanisms other than those with which we are now familiar; and in this search for other possible mechanisms of immunity it is important that obscure forms of native as well as acquired resistance be subjected to close examination, for our present information renders it altogether likely that the basic protective mechanisms in both types of resistance are of the same fundamental nature. It was for this reason that we engaged in the present study, which is concerned with the debated problem of the mechanism of the native immunity of the rabbit to the Type III pneumococcus.

Although rabbits are highly susceptible to virulent Type I pneumococci, they are markedly resistant to most virulent strains of Type III pneumococci. In most instances of native immunity it has been possible to show that the resistance is due principally either to the fact that the bacteria are readily phagocytized by the leucocytes, or that the animal's normal temperature is incompatible with the proliferation and life of the bacteria. The native immunity of the rabbit to the Type III pneumococcus is an exception to this rule, and has been regarded as peculiar for the reason that the bacteria grow well at the normal body temperature of the animal, and both *in vivo* and *in vitro* studies have, up to the present, failed to reveal any appreciable phagocytosis of the bacteria. This matter has been studied

particularly by Singer and Adler (3) and by Tillett (4), and these investigators were unable to find any evidence that the phagocytes are active in the destruction of Type III pneumococci in the normal rabbit that withstands the infection. Singer and Adler (3) observed phagocytosis by the local macrophages, but to no appreciable degree by the polymorphonuclear leucocytes, when Type III pneumococci were injected directly into the bone marrow of rabbits that had been previously immunized by vaccination, and they believed this to be due to an alteration of the macrophages themselves (an "Umstimmung" of the reticulo-endothelial system) rather than to antibody formation (opsonin). Without entering here into a critique of those experiments on vaccinated animals, with the conclusions of which we are unable to agree, it may be said only that they throw no light upon the problem before us, namely, the mechanism of the normal rabbit's resistance to this bacterium. Tillett (4), unable to observe phagocytosis of the bacteria by the leucocytes of the normal rabbit, concluded that the animal must possess some other undetermined mechanism capable of destroying the Type III pneumococcus.

Since the native resistance of the rabbit to the Type III pneumococcus appeared, from the work of these previous investigators, to represent an instance in which some factor other than those familiar in native immunity might be in operation, we were attracted to a study of the phenomenon with a view toward determining whether the known mechanisms of immunity are actually inadequate to account for this particular instance and, if so, to attempt to discover what the undetermined "x factor" might be.

Our interest in this problem was first aroused several years ago when, during the course of experiments on the relation of phagocytes and antibody to resistance, we found that, in spite of the reported failure of the leucocytes to play a rôle in the native immunity of the rabbit to the Type III pneumococcus, these bacteria proliferated progressively to colossal numbers in the tissues of rabbits deprived of leucocytes, whereas in animals possessing leucocytes they disappeared completely after some preliminary multiplication (1). This led us to believe that the phagocytes must play a rôle in this instance of native immunity, in spite of reports to the contrary. Pursuing the study of this problem, two years ago we presented before the

Johns Hopkins Medical Research Society (5) the results of experiments in which it was demonstrated that phagocytosis of the Type III pneumococcus does occur under proper conditions in rabbit serum-leucocyte mixtures; and, in addition, that strains of Type III pneumococci to which the rabbit is natively resistant are markedly susceptible to the degree of heat represented by the rabbit's fever temperature. It is the purpose of the present paper to set forth the details of those experiments, and to demonstrate more directly their significance by means of appropriate *in vivo* studies.[1]

EXPERIMENTS

Strains used in the present study. In these studies we have used eight strains of Type III pneumococci (designated as CM, O, RM, SM, SqM, SP, SV, and XM). All of these strains were well encapsulated, produced mucoid colonies on blood agar, were agglutinated specifically by Type III antiserum, and showed no cross agglutination with Type I or Type II antiserum. Three of the 8 strains (RM, SP, XM) were freshly isolated from fatal human infections; the remaining strains were older cultures derived from human infections. The strains were repeatedly typed during the experiments to check the strictness of their type-specificity, for it has been our experience that Type III pneumococci are, in general, decidedly less stable than Type I pneumococci. *In vitro* many of these strains tend to dissociate partially into rough variants. It is essential, therefore, to maintain a constant vigilance over their characteristics by cross agglutination tests and by observations on colony morphology and degree of capsule formation. Frequent animal passage, of course, tends to ensure their smooth qualities. With proper precautions pure smooth cultures can be maintained for extended study.

Of the eight strains used in this investigation only one was virulent for the rabbit to any appreciable degree. This was the Rockefeller Institute SV strain, for a culture of which we are indebted to Dr. O. T. Avery. Even this virulent strain has at intervals exhibited

[1] Results of these *in vivo* studies were presented at the 1936 meeting of the American Association of Immunologists (Jour. Immunol., 1936, xxx, 389). At the same meeting Enders and Shaffer (*ibid.*, p. 382) reported *in vitro* studies which are in close agreement with our own observations.

alterations both in its virulence and in its resistance to phagocytosis. Tillett (4), who studied this strain several years ago, likewise noted attenuation at times when it was kept out of the animal body for even several days. The observations on this strain reported here refer to its properties in its rabbit-virulent state. All of the strains used in this investigation were virulent for mice.

Throughout the experiments the organisms were grown in beef infusion broth enriched by the addition of 5% defibrinated rabbit's blood. Rabbit's blood agar was used for plating.

Comparison between the activity of the Type III and the Type I pneumococcus in the rabbit. When 0.1 cc. of a 7 to 8 hour culture, or of a 1–10 dilution of culture, of any of the seven strains to which the rabbit is natively resistant is injected intracutaneously on the upper part of the shaved flank, there appears after several hours an erythematous, oedematous lesion which spreads gradually downward over the side in a band 2 to 5 cm. wide, often reaching the midline of the abdomen within 24 hours. Purpuric spots or more extensive haemorrhages commonly appear in the lesion (Fig. 1). The various strains differed somewhat in the severity of the lesion produced by a given dose. In Table IV they are arranged according to the severity of the lesion produced by a given dose of each. As will be pointed out later, there is a definite degree of individual variation among rabbits, and this table was prepared from the averages of a large number of intracutaneous infections carried out during the progress of this work. There is no significant difference between the general appearance of the lesion produced by the Type III pneumococcus to which the rabbit is resistant and that produced by the Type I pneumococcus to which the animal is highly susceptible, but a far smaller dose of virulent Type I pneumococci will suffice to produce a lesion of equal size, while decreasing the dose of the Type III results in great diminution of the size of the lesion. Furthermore, a rabbit with a similar lesion produced by virulent Type I pneumococci will invariably die with massive septicaemia 24 to 48 hours after the injection of the bacteria, whereas the invasion of the blood stream by rabbit-resistant strains of the Type III pneumococcus is always relatively slight, and the local lesion begins to regress after two or three days. Regression often begins in the upper portion of the lesion at the site of injec-

tion and progresses downward until, after a lapse of time varying usually between 4 and 6 days in the case of most of these strains, the erythema and oedema have disappeared completely, the blood is sterile and the rabbit remains well. The relative resistance of the rabbit to the Type III pneumococcus can be appreciated from the fact that the intracutaneous injection of one-millionth the dose of virulent Type I pneumococci regularly produces a lesion of the same size with a fatal outcome. Indeed, 5 to 10 cc. of a heavy blood-broth culture of various mouse-virulent Type III strains to which the rabbit is natively resistant can be injected into the blood stream or pleural cavity of the rabbit without a fatal outcome, whereas the injection of only a few diplococci of virulent Type I pneumococci will cause a fatal infection.

Microscopic study of the local lesions produced by the intracutaneous injection of Type III pneumococci shows no appreciable difference in reaction from those caused by the Type I pneumococcus. Lesions removed at various intervals for study show that in both there is an early outpouring of inflammatory oedema and a progressively increasing accumulation of polymorphonuclear leucocytes. In both there occurs a proliferation of the bacteria during the first 24 hours. However, at every stage after the first few hours the number of Type I pneumococci in the tissues is greater than that of the Type III; and whereas the rabbit infected with Type I dies while the lesion is swarming with the bacteria, the Type III organisms of rabbit-resistant strains after reaching considerable numbers during the first 24 hours begin to decrease in numbers, until after two to three days they become difficult to find and eventually disappear completely. The decidedly more active proliferation of the Type I pneumococcus in the rabbit is also readily observable if equal amounts of Type I and Type III pneumococci are injected into the pleural cavities of different rabbits and smears and poured plates made of the exudates at intervals, the factor of differences in dilution by exudate being controlled by collecting and measuring the entire exudate in each case. Under such conditions the growth of the Type I pneumococcus is found to outstrip greatly that of the Type III from the beginning, until colossal numbers are produced and the animal dies with overwhelming septicaemia. The Type III organisms also increase

markedly in numbers, but after about 12 hours they begin to decrease greatly and within 18 to 24 hours have usually become very difficult to find in smears, and complete sterilization eventually occurs. It is not only in the body of the rabbit that the Type I grows better than the Type III. Inoculated in equal doses into defibrinated rabbit blood, rabbit serum, or the fluid from a sterile inflammatory pleural exudate, plates made at frequent intervals show that the Type I has consistently a more rapid and abundant growth than the Type III pneumococcus, whether incubated at 37° or at higher temperatures.

From these observations three pertinent facts emerge. First, Type III pneumococci to which the rabbit is natively resistant are able to proliferate readily for a time in the body of the rabbit. The native immunity of the rabbit is, therefore, not due to any inability of the animal's body to support the growth of these bacteria. Second, the growth of the Type III pneumococcus is less rapid in the rabbit than is that of the Type I to which the animal is highly susceptible. And finally, whereas the growth of the Type I pneumococcus continues progressively up to the death of the animal, after a lapse of some hours the proliferation of the Type III ceases, and the bacteria die and are destroyed. The problem, therefore, is to determine what it is that stops the initial proliferation of the Type III pneumococci and brings about their destruction.

The rapid development of resistance to infection. Since it is clear that after the bacteria have multiplied for some hours in the tissues something happens in the body of the rabbit which prevents their further growth and brings about their death, it was desirable to learn whether this newly acquired power to prevent the growth and survival of the bacteria is a property of the entire body or is limited only to the immediate site of the lesion. In order to determine this point rabbits were infected intracutaneously on one side of the body with 0.1 cc. of a 1–10 dilution of culture of rabbit-resistant strains, and at the end of 48 hours the same amount of a fresh culture of the same age was injected into the skin of the opposite flank. The difference between the results of the two injections was indeed astonishing. Whereas the first injection always produced the characteristic extensive lesion, the second injection on the opposite side produced a markedly smaller lesion which remained limited in size, regressed and disappeared.

The time between infection and regression was regularly less than that required for the regression of the first lesion, and while the lesion of first infection usually became purpuric it was very exceptional for haemorrhage to appear in the smaller second lesion.

When a sufficient number of animals had been infected in this manner upon the opposite side of the body to make it clear that at the end of 48 hours after a first infection the rabbit has acquired the power to restrict greatly a second infection, we sought to determine how soon following the initiation of a first infection this heightened resistance to further infection makes its appearance. To this end, rabbits were infected on the opposite flank 6, 12, 24 and 36 hours following the first infection, the dose of the second injection always being the same amount of a culture of the same strain and same age as that of the first injection. These experiments showed plainly that at 24 hours the increased resistance was present in as high a degree as at 48 hours. At 12 hours some of the rabbits showed a distinct resistance to the second infection but the restriction of the lesion was not as constant as at 24 hours. Figures 1 and 2 show the marked difference between the typical lesion of first infection and that of a second infection initiated 24 hours after injection of the bacteria of the first infection. Table I lists the difference between the reaction of a series of rabbits to first and second infection.

These experiments demonstrated clearly that within the first 24 hours after the intracutaneous infection the rabbit acquires a marked ability to limit a second infection at a different site. Since the second lesion is much smaller than the first, rarely becomes purpuric and regresses in a shorter time than the first lesion, it is apparent that the multiplication of the bacteria in the second lesion must be inhibited and their destruction effected more rapidly than in a first infection and, indeed, bacterial stains of the tissues and cultures of the lesions show this to be the case.

For this purpose rabbits were infected intracutaneously with 0.1 cc. of a 1–10 dilution of culture of strain SM. Forty hours later they were infected on the opposite side with the same amount of a fresh culture and at the same time normal animals were injected with the same dose of the same culture to provide lesions of first infection of the same age. Portions of the lesions of second infection and of the lesions of first infection produced in the normal controls were removed

TABLE I

Rapid development of resistance to infection in rabbits injected intracutaneously with Type III pneumococci

NORMAL RABBIT	FIRST INJECTION	LARGEST SIZE OF LESION	INJECTION ON OPPOSITE SIDE	LAPSE OF TIME BETWEEN 1ST AND 2ND INJECTION	TEMPERATURE AT TIME OF 2ND INJECTION	LARGEST SIZE OF 2ND LESION
		cm.		hrs.		cm.
8	0.1 cc. 1–10 dil., Type III	13 x 3*	0.1 cc. 1–10 dil., Type III	48	106°	4 x 2
13	0.1 cc. 1–10 dil., Type III	15 x 6*	0.1 cc. 1–10 dil., Type III	48	107°	3 x 2½
14	0.1 cc. 1–10 dil., Type III	15½ x 4½*	0.1 cc. 1–10 dil., Type III	48	104.5°	4 x 2
60	0.1 cc. 1–10 dil., Type III	15 x 5*	0.1 cc. 1–10 dil., Type III	48	104.2°	7 x 2
61	0.1 cc. 1–10 dil., Type III	15 x 3½*	0.1 cc. 1–10 dil., Type III	48	103.2°	9 x 5
62	0.1 cc. 1–10 dil., Type III	13 x 2½	0.1 cc. 1–10 dil., Type III	48	103.5°	4 x 2
67	0.1 cc. 1–10 dil., Type III	10½ x 3	0.1 cc. 1–10 dil., Type III	48	103°	13 x 4
3	0.1 cc. 1–10 dil., Type III	15 x 3	0.1 cc. 1–10 dil., Type III	48	105.8°	6 x 3½
9	0.1 cc. 1–10 dil., Type III	16 x 3*	0.1 cc. 1–10 dil., Type III	36	105.5°	V. sl.
15	0.1 cc. 1–10 dil., Type III	15 x 6*	0.1 cc. 1–10 dil., Type III	36	104.6°	3 x 3
16	0.1 cc. 1–10 dil., Type III	15 x 11*	0.1 cc. 1–10 dil., Type III	36	104.5°	4 x 2*
10	0.1 cc. 1–10 dil., Type III	15 x 3	0.1 cc. 1–10 dil., Type III	24	104.5°	4 x 1½
17	0.1 cc. 1–10 dil., Type III	9½ x 4*	0.1 cc. 1–10 dil., Type III	24	104.5°	4½ x 2½
18	0.1 cc. 1–10 dil., Type III	15 x 7*	0.1 cc. 1–10 dil., Type III	24	104.2°	4 x 2
19	0.1 cc. 1–10 dil., Type III	15 x 6*	0.1 cc. 1–10 dil., Type III	12	103.7°	12 x 2½
20	0.1 cc. 1–10 dil., Type III	15 x 8*	0.1 cc. 1–10 dil., Type III	12	104.3°	7 x 5*

* Lesion purpuric.

immediately after injection of the bacteria and 6, 12, 24, 48, and 72 hours later for histological and bacteriological study. At each period the following procedure was carried out.

A rectangular area 1 x 1½ in. was carefully marked out on the skin at the site of the lesion with india ink, the center of the upper line of the rectangle being determined by the marked point at which the injecting needle had penetrated the skin. The rectangle was then divided into two equal parts by a central vertical line of india ink. The area was washed with soap and water, followed by alcohol and ether. Under aseptic precautions the rectangular area of skin was excised and cut into halves along the central ink line. Half of the lesion was fixed in Zenker-formol for histological study. The other half was minced with scissors and thoroughly ground in a mortar with sterile sand in 5 cc. of broth. The fluid was transferred to a sterile centrifuge tube and rotated at low speed for 1 minute. Poured blood-agar plates were made at once for colony counts, using 0.1 cc. of the undiluted supernatant fluid as well as dilutions of 1–100 and 1–1000.

The colony counts in these studies showed an increase in pneumococci in the lesions of both first and second infection at the end of 6 hours. At 12 hours the bacteria had increased still further in the lesions of first infection, but in the lesions of second infection at this time there was either no further increase or even a beginning decrease in numbers. At 24 hours the number of bacteria had markedly decreased in the lesions of second infection, the plates of the undiluted fluid showing only one or two colonies as contrasted with the great number of colonies (4+) obtained from the undiluted fluid of the lesions at 12 hours. In the lesions of first infection at this time a decrease in the number of bacteria had occurred, but it was far less of a decrease than in the lesions of second infection. At 48 and 72 hours the plates of the undiluted fluid from the lesions of second infection showed only an occasional colony or none at all, while colonies were still plentiful in the lesions of first infection though markedly decreasing in numbers. The histological sections of the lesions removed at the various intervals showed no difference between the lesions of first and second infection other than a more progressive and extensive inflammation in the lesions of first infection, in which after 6 hours bacteria were far more numerous than in the lesions of second infection. In the latter lesions it was extremely difficult or impossible to find bacteria after 24 hours, whereas many could readily be found in the lesions of first infection at this time.

From these studies it is clear that within a matter of hours after

intracutaneous infection with rabbit-resistant strains of the Type III pneumococcus the rabbit acquires the power greatly to restrict the growth and to bring about the destruction of the bacteria. This power is, however, not sufficiently great at any period to inhibit completely the growth of the bacteria of second infection in the infecting dose used in these experiments. Injected in this amount, the bacteria multiply during the first few hours, but their proliferation is less active, it ceases sooner, and the bacteria are destroyed much earlier than are those in the lesion of first infection. What is the mechanism by means of which this remarkable, rapidly acquired power of resisting further infection is effected?

The mechanism of the rapidly acquired resistance. The very brief time required for its appearance made it unlikely that the markedly enhanced resistance to infection could be due primarily to specific acquired immunity, and it may be stated briefly that in subsequent experiments we were unable to demonstrate any protective or other antibody in the blood of rabbits taken at 24, 36 or 48 hours after a first infection, at which periods the animals were highly resistant to a second infection. But more important, it was soon found that in the great majority of cases the animals are no longer resistant to a second infection after a first infection has entirely healed. Infected with the same dose 10 days to several weeks after a first infection they develop lesions quite as large as the original ones. It is only in an occasional rabbit that any resistance, as evidenced by restriction of the size of the lesion, can be detected after the lesion of first infection has healed. It is clear that a single intracutaneous infection of this nature rarely confers any specific resistance to subsequent infection. Obviously, therefore, the enhanced resistance in the great majority of the animals under discussion is dependent upon conditions that are present only during the stage of activity of the first lesion. We therefore turned our attention to non-specific factors associated with infection.

In the first place, it may be stated that the skin of the flank opposite to that on which the first infection is established is perfectly normal grossly and histologically at the time when the second infection is induced there. We studied this matter carefully for there was the possibility that the inflammation on one side of the body might

have extended histologically to the opposite side and so have produced an area of "prepared inflammation" such as is well known to be resistant to infection with various bacteria. Histological study shows, however, that the tissues of one flank remain perfectly normal throughout the course of the most extensive primary lesion on the other flank. Furthermore, we found that if the bacteria of first infection were injected intravenously instead of intracutaneously, thus avoiding any cutaneous lesion of primary infection, resistance to subsequent intracutaneous infection appeared quite as early and in as high a degree as when the primary infection was an intracutaneous one.

It seemed conceivable that the heightened resistance that appears during the course of the primary lesion might be due to leucocytosis providing a more abundant supply of phagocytes to attack the bacteria of second infection, and this particularly since we had found that Type III pneumococci grow more freely in animals deprived of leucocytes (1). Studies of this point showed, however, that the ability to resist a second infection bore no constant relation to the degree of leucocytosis. Indeed, some animals had a perfectly normal leucocyte count at the time of second infection even in the face of large primary lesions, and these animals were as resistant to the second infection as were those with leucocytosis.

The important rôle of fever temperature. A third non-specific factor requiring consideration was temperature, and this we found to be of very great importance. The normal body temperature of the rabbit is considerably higher than that of the human being, ranging usually between 102° and 103°. Within a few hours after infection the temperatures rises usually to 104° to 106°. Since we were unable to find any determinations of the heat sensitivity of the Type III pneumococcus in bacteriological treatises, we carried out *in vitro* experiments two years ago which revealed a marked susceptibility of seven of our eight strains to the degree of heat represented by the rabbit's fever temperature (5) (6).

In these experiments, 0.1 cc. of a 1–1,000,000 or 1–100 dilution of a 7 to 8 hour blood-broth culture of the various strains was inoculated into tubes of rabbit serum, defibrinated rabbit blood, heparinized rabbit plasma or blood-broth, and the tubes incubated at 37°, at 40°

and at 41°C. Poured plated were made at frequent intervals for colony counts. Many experiments of this type were carried out, and they showed clearly that all of our strains except SV were markedly susceptible to temperatures of 40°–41°, the inhibitory effects being greater at 41° but definite at 40°. Neither the SV strain nor either of two Type I strains were appreciably affected by exposure to these temperatures. The SV strain, it will be remembered, is the only one of our Type III strains that possesses any appreciable degree of virulence for the rabbit, and the Type I strains had a high degree of rabbit virulence. The seven strains that were inhibited *in vitro* at the rabbit's fever temperature differed somewhat in the degree of their sensitivity to that temperature. At 40°–41° their growth was distinctly inhibited as compared with that at 37°, but colony counts made at intervals following incubation showed that the inhibition appeared earlier in some of the strains than in others. The heat sensitivity manifested itself in a more important way than inhibition of proliferation, however. Complete sterilization occurred at 40°–41°, whereas the bacteria proliferated progressively and remained alive during the same period at 37°. The time required for sterilization to occur at 40°–41° was also different for different strains, as can be seen in Table II.

It may be noted that the character of the medium in which the bacteria were placed during the heat sensitivity tests influenced both the degree of inhibition of growth and the rapidity of sterilization. In general, the growth of the bacteria was less inhibited and they survived longer at 40°–41° in media which supported their growth best at 37°. Thus, both the inhibition of growth and final sterilization appeared earlier at 40°–41° in serum than in defibrinated blood, while blood-broth permitted a better growth at 40°–41° than did defibrinated blood. A protocol illustrating the degree to which the various strains were inhibited at 40° in serum, defibrinated blood and blood-broth is shown in Table II.

These experiments bring to light a very important fact relating to the mechanism of the native immunity of the rabbit to the Type III pneumococcus. It becomes evident that, *in vitro* at least, the fever temperature of the rabbit is incompatible with the free proliferation and survival of our strains to which the animal is natively resistant,

while the only one of our strains to which the rabbit is susceptible (SV) is able to proliferate and to survive at the animal's fever temperature. The Type I pneumococcus, likewise, to which the rabbit is susceptible, was unaffected by the rabbit's fever temperature. It seems clear, therefore, that the degree of fever which develops in the rabbit several hours after infection must play a very important rôle in the native resistance of the animal to these strains of Type III pneumococci.

The effect of elevating the temperature prior to infection. *In vitro* effects, although providing important leads, cannot be assumed to be operative under *in vivo* conditions without direct test. Therefore,

TABLE II

*Inhibition of Type III pneumococci at 40° in different media**

STRAIN	RABBIT SERUM				DEFIBRINATED RABBIT BLOOD				BLOOD BROTH			
	3 hrs.	6 hrs.	11 hrs.	24 hrs.	3 hrs.	6 hrs.	11 hrs.	24 hrs.	3 hrs.	6 hrs.	11 hrs.	24 hrs.
SV	110	++	++	0	++	++++	∞	+++	++	++++	∞	++++
XM	9	0	0	0	37	++	++++	0	57	++	++++	0
RM	4	0	0	0	27	132	12	0	10	70	++	0
O	12	1	0	0	9	0	0	0	82	++	++	0
SP	6	0	0	0	18	30	9	0	9	10	2	0
SM	6	0	0	0	5	0	0	0	70	+	55	0
SqM	4	0	0	0	12	0	0	0	7	10	4	0
CM	7	0	0	0	5	0	0	0	21	18	0	0

* 2 cc. media inoculated with 0.1 cc. 1–1 mil. dilution of culture.
Figures represent colony counts of 0.1 cc. on blood agar plates.

while the temperature effects as revealed so clearly *in vitro* seemed adequate to explain, in part at least, the inhibition of a second infection induced during the fever period of the first infection, it seemed desirable to test the inhibitory effect of fever temperature *in vivo* under conditions independent of any previous infection. To this end, normal rabbits were placed in an incubator room, the temperature of which was maintained at 37° by a thermostat. The animals were liberally supplied with food and water. Placed in an environment of 37°C. the rabbit is unable to maintain its normal degree of body heat, and within a few hours its temperature rises to 104°–106°F. and remains at the elevated level. 12 to 48 hours after the animals had

been placed in the incubator room they were infected intracutaneously with 0.1 cc. of a 1–10 dilution of a 7-hour culture of the various strains of Type III pneumococci. The results were most striking. All strains, except SV which had been more heat resistant *in vitro*, invariably produced lesions which were markedly smaller and regressed earlier than those in control rabbits given the same dose at the same time and left at room temperature. The inhibition of the infection in these animals was, in general, more marked than was the inhibition of a second infection induced during the febrile period of a first infection. Figure 3 is a photograph of a typical restricted lesion developing in a rabbit with an elevated temperature produced by sojourn in the incubator room. Table III shows the comparison between the size of the lesions in heated and in control rabbits infected with several different strains. The same inhibition of infection by elevated temperature was obtained with other rabbit-resistant strains.

In other experiments rabbits with temperatures elevated to 105°–106° by sojourn in the incubator room were infected intrapleurally with 1.0 cc. of a 7-hour culture of strain SM, and the course of the infection was followed in comparison with that in controls given the same dose but kept at room temperature. Smears, cultures and colony counts were made of the pleural exudate removed at intervals following infection. Correction for differences in dilution produced by differences in the amount of exudate formed in individual rabbits was made by collecting and measuring all of the exudate at each interval, using a series of rabbits instead of making repeated observations on single individuals. These experiments showed that in the heated animals the initial proliferation of the bacteria was checked, and that they disappeared earlier from the pleural cavity than in the controls kept at room temperature.

The above experiments make it perfectly clear that the fever temperature of the rabbit is a highly important factor in the native resistance of the animal to the Type III pneumococcus. Both *in vitro* and *in vivo* a temperature of 40°–41°C. exerts a marked inhibitory effect upon most strains of these bacteria that we have examined. Indeed, only one of our eight strains (SV) was resistant to this degree of temperature, and this was the only strain that possessed any appreciable degree of virulence for the rabbit.

It was stated above that two Type I strains, highly virulent for the

rabbit, were tested and found not to be sensitive to 40°–41° temperature *in vitro*. One of the Type I strains was injected intracutaneously (0.1 cc. 1–50,000 dilution) into a rabbit with fever produced by a Type III lesion on the opposite side of the body, and also into a rabbit with temperature elevated by sojourn in the incubator room.

TABLE III

Inhibition of Type III pneumococcal lesion produced by elevating the temperature in an incubator room

RABBIT	TIME IN INCUBATOR BEFORE INFECTION	TEMPERATURE AT TIME OF INFECTION	INFECTING DOSE	STRAIN	LARGEST SIZE OF LESION	SURVIVAL
	hrs.				*cm.*	
71	24	103.8°	0.1 cc. 1–10 dil.	SP	4 x 3	S
72	12	106°	0.1 cc. 1–10 dil.	SP	1 x 1	S
73	12	105°	0.1 cc. 1–10 dil.	SP	2½ x 2½	S
Control		102.8°	0.1 cc. 1–10 dil.	SP	13 x 3	S
Control		103°	0.1 cc. 1–10 dil.	SP	13 x 5	S
Control		103.3°	0.1 cc. 1–10 dil.	SP	8 x 2½	S
81	12	105°	0.1 cc. 1–10 dil.	SqM	3½ x 3½	S
82	12	105.5°	0.1 cc. 1–10 dil.	SqM	v. sl.	S
Control		102.3°	0.1 cc. 1–10 dil.	SqM	12 x 4	S
Control		103°	0.1 cc. 1–10 dil.	SqM	12 x 3	S
77	24	104.5°	0.1 cc. 1–10 dil.	SM	5 x 2	S
78	48	104.5°	0.1 cc. 1–10 dil.	SM	2 x 2½	S
Control		102.5°	0.1 cc. 1–10 dil.	SM	15 x 6	S
Control		101.5°	0.1 cc. 1–10 dil.	SM	12 x 6	S
89	24	104.3°	0.1 cc. 1–10 dil.	SV	11 x 6	D 80 hrs.
90	24	104.3°	0.1 cc. 1–10 dil.	SV	11 x 5	D 56 hrs.
Control		102.5°	0.1 cc. 1–10 dil.	SV	15 x 6	D 72 hrs.

In each case the usual large, purpuric lesion developed and the infection progressed to a fatal outcome.

Since all of our eight strains of Type III are virulent for the mouse, it is interesting to note that the normal temperature of the mouse is several degrees lower than that of the rabbit, averaging about 98° and, furthermore, that following intraperitoneal infection with these organisms the temperature rises usually surprisingly little, rarely in our experience being sustained for any length of time above 101°

and not infrequently failing to reach 100°. The fever temperature of this animal, for which these bacteria are highly virulent, is, therefore, within the range of heat at which the organisms proliferate freely. We have carried out four experiments in which the attempt was made to protect mice against the various strains by raising their temperature in the 37°C. incubator room. Forty mice were used in these experiments. The temperature of the mice was rarely raised to even 103°F. in the incubator—a level too low to affect appreciably the Type III pneumococcus. In most cases the temperature was only 99°–102°. Further attempts to protect mice by elevating their temperature in an environment maintained at a higher temperature are in progress. It may be stated that repeated tests have shown that the growth of these bacteria is readily suppressed *in vitro* in the serum of the mouse when the temperature is elevated to 40°–41°C.

The inhibitory effect produced by elevating the rabbit's temperature in the incubator room is, as far as we have been able to determine, referable directly to the temperature and not to other bodily alterations. That this is the case, is indeed, indicated by the fact that the inhibition occurs at 40°–41° *in vitro* where other factors that might make their appearance in the living animal are excluded. We have found no change in the leucocyte count in most rabbits heated in the incubator room. Furthermore, defibrinated blood and serum obtained from rabbits during the elevation of temperature produced by sojourn in the incubator room had no more bacteriostatic or bactericidal effect upon the Type III pneumococcus than had the blood or serum of normal rabbits.

In vitro phagocytosis of the Type III pneumococcus. As striking as was the protective effect of fever temperature, as demonstrated by the above experiments, there were reasons for believing that this was not the entire story of the resistance of the rabbit to the Type III pneumococcus. In the first place, the lesions usually become teeming with pneumococci as a result of the proliferation of the organisms during the pre-febrile period, and *in vitro* not all of the strains would be killed off completely at 40°–41° when heavy inocula were used. When 1 cc. of a straight culture was injected into the pleural cavity of a normal rabbit the proliferation of the bacteria usually led to the production of colossal numbers of organisms within the first few pre-

febrile hours, after which the bacteria began to disappear so rapidly that at the end of eight to ten hours more they might be difficult to find in smears; and if the animal's temperature was raised by placing it in the incubator room before injecting the bacteria the virtual disappearance of the organisms took place even more rapidly. It seemed that some mechanism other than lysis might be in operation to account for the rapid disappearance of the organisms, for in our studies of immunity in the absence of leucocytes we had found that Type III pneumococci increase to great numbers in the tissues of rabbits deprived of leucocytes until the animal dies with heavy septicaemia (Figures 14 and 15). While the temperature of the benzol-treated animals was usually lower following infection than that of previously normal animals, nevertheless, in animals receiving the same amount of benzol, and likewise having a lower temperature after infection than controls but in which, because of individual variation, the leucocytes were not sufficiently depressed to prevent some emigration at the site of infection, a striking disappearance of the pneumococci was observed to occur wherever clumps of leucocytes had accumulated in the tissues. Since phagocytosis plays so important a rôle in the disposal of all other types of pneumococci by the immune body, whether the immunity be native or acquired, it seemed reasonable to suspect that it was probably playing a rôle in immunity to the Type III as well, particularly in view of the above mentioned considerations. In spite of the failure of previous investigators to detect phagocytosis in this instance of native immunity, we turned, therefore, to a closer examination of this point.

In our first experiments we carried out the usual phagocytic test by mixing leucocytes, obtained from centrifugalized 18 hour aleuronat pleural exudates, with fresh rabbit serum and the various strains of Type III pneumococci, incubating the mixtures at both 37° and 40°, and staining smears for bacteria at the end of 30 minutes and 1 hour. The pneumococci used were blood-broth cultures grown from mucoid colonies, and also organisms taken directly from mucoid colonies on blood-agar plates. Mixtures containing readily phagocytizable, non-encapsulated rough pneumococci were set up as controls for the activity of the leucocytes. The results of these experiments were in agreement with those of Tillett (4)—*i.e.*, there was no appreciable

phagocytosis of any of the strains of the encapsulated pneumococci, while the rough pneumococci were avidly phagocytized. In view of Singer and Adler's conclusions regarding the efficacy of mononuclear phagocytes in ingesting the Type III pneumococci in vaccinated rabbits (3) we tested the activity of exudates composed chiefly of macrophages and derived from non-vaccinated, normal rabbits and found them to exhibit no more phagocytosis within an hour than did the polymorphonuclear exudates.

Such a test, however, fails in a very important respect to reproduce the conditions that obtain in the infected body. In the latter case, the bacteria proliferate in the body fluids of the rabbit for at least several hours before any appreciable number of leucocytes have emigrated to the site to attack them. It therefore seemed advisable to continue the *in vitro* observations for a period longer than that usually regarded as sufficient for a phagocytic test. The incubation of the serum-polymorphonuclear-leucocyte-bacteria mixtures was therefore continued in subsequent experiments and it was found that after a lapse of time varying between 8 and 24 hours phagocytosis of the Type III organisms occurred. Figure 6 illustrates *in vitro* phagocytosis of RM, a highly encapsulated strain. The various strains differed both in the time required for phagocytosis to begin and in the degree to which phagocytosis was present at a given period. These characteristics were not always constant for a given strain in different experiments, but many such experiments have permitted the arrangement of the different strains according to the approximate readiness with which they become phagocytized, as shown in Table IV. The phagocytosis of the rabbit-virulent SV strain occurred later and to a much lesser degree than that of the other strains. Frequently, scarcely any phagocytosis of this strain was observed during the entire period of incubation.

It was soon found that older cultures were phagocytized after a shorter period of incubation with the leucocytes than were young cultures. Thus, the organisms from 18 to 24 hour cultures were phagocytized more rapidly than were those of a 7-hour culture. While the rapidity and degree of phagocytosis varied in different experiments, even in the case of a given strain, marked phagocytosis was observed often enough to convince us that under proper condi-

tions phagocytosis of Type III pneumococci does occur and has to be reckoned with in analysis of the native immunity of the rabbit to these organisms. We therefore turned to a study of phagocytosis *in vivo*.

The phagocytosis of the Type III pneumococcus in the body of the rabbit. In order to study the fate of the bacteria *in vivo*, normal rabbits were injected intrapleurally with 1.0 cc. of a 7 to 8 hour culture of the various strains, and the resulting exudate was removed at intervals and stained with Gram's and other bacterial stains suitable for the study of phagocytosis. The exudation of fluid and emigration of leucocytes into the pleural cavity began shortly after the injection,

TABLE IV

*Comparative characteristics of 8 strains of Type III pneumococci**

SENSITIVITY TO 40°–41°	SIZE OF CAPSULE	TENDENCY TO LOSE CAPSULE AND BECOME PHAGOCYTIZABLE	SEVERITY OF LOCAL LESION
CM	XM	O	SV
SqM	SV	CM	XM
SM	RM	SqM	SM
SP	SP	SM	SP
O	SM	RM	RM
RM	SqM	SP	SqM
XM	CM	XM	CM
SV	O	SV	O

* The strains are arranged from above downward in decreasing order of degree in regard to the respective headings.

and at the end of five hours from 5 to 10 cc. of leucocyte-rich exudate had usually accumulated in the cavity. During the first 6 hours following the injection the bacteria multiplied greatly and during this time no appreciable degree of phagocytosis of any of the strains could be observed. At periods between 6 and 18 hours after injection of the bacteria, however, phagocytosis of the strains other than SV became plainly apparent and increased continually in amount, while concomitantly the number of free pneumococci decreased until, between 18 and 24 hours following infection, extracellular organisms were rare, and eventually none could be found at all. In the earlier stages of the phagocytic process well formed cocci predominated in the polymorphonuclear cells. In the later hours of the infection

partly digested forms were more prominent in these cells, and with sterilization of the exudate there was complete intracellular digestion and disappearance of all fragments. When phagocytosis was well under way many leucocytes could be seen containing from several to twenty or more cocci. Occasional mononuclear phagocytes containing cocci could be seen, but the pneumococci had usually disappeared from the exudate before any appreciable number of mononuclear cells had made their appearance. Figures 4, 5 and 7 show the phagocytosis of strain SM at 18 hours and O at 12 hours after infection. Phagocytosis of the rabbit virulent SV strain was relatively negligible at all periods, and the bacteria increased progressively up to the death of the animal.

Degeneration of the capsule of the Type III pneumococcus in the body of the rabbit. The above *in vitro* and *in vivo* studies made it clear that, contrary to the reports of previous investigators, phagocytosis of Type III strains to which the rabbit is natively resistant does play a rôle in disposing of the bacteria. Ordinarily, in phagocytic tests, if phagocytosis of bacteria occurs at all, it can be observed within a matter of minutes and certainly within the first hour. In the present instance, however, this is not the case. A period of several hours at least, and often much longer, has been required for the appearance of outspoken phagocytosis. Once phagocytosis of these strains has begun *in vivo* it may occasionally progress with such rapidity that within a few hours only minute fragments of partly digested cocci remain in the leucocytes. Observations made shortly after this may show practically no cocci either intra- or extracellularly. In such cases it is important that observations be made at frequent intervals, or the entire process may be missed. In other cases, however, there is a gradual and continual phagocytosis, the individual leucocytes containing only one or two diplococci and fragments instead of becoming packed with them.

The question arises at once why it is that if phagocytosis occurs at all, it does not take place during the first 30 minutes to an hour, as in the case of other phagocytizable bacteria. In the case of the pleural exudate it might be thought that the great number of bacteria present by the time that appreciable numbers of leucocytes have emigrated might conceivably liberate enough soluble specific

substance to inhibit phagocytosis. The fact that phagocytosis does eventually begin in the presence of great numbers of the organisms argues against this possibility, but to test it we injected non-encapsulated (rough) pneumococci together with the usual dose of the encapsulated organisms. Smears of the exudate showed that the non-encapsulated forms were avidly phagocytized during the early period in which no phagocytosis was demonstrable in controls injected with the encapsulated strains alone.

Since both *in vitro* and within the body, the encapsulated strains to which the rabbit is natively resistant proliferate for some hours in contact with many leucocytes and yet remain unphagocytized, and then become phagocytizable, it is evident that some change must take place in the bacteria to permit their phagocytosis. Since it is well known that encapsulated pneumococci resist phagocytosis, while the non-encapsulated, rough forms are readily phagocytizable, a loss of the capsule would be the most obvious explanation of the delayed phagocytosis, and this is precisely what occurs. In the beginning of our work with Type III pneumococci we observed that cultures 18 to 24 hours old usually showed a much greater variation in the size of the capsules of the individual cocci than did young cultures. In the older cultures, by the side of well-encapsulated cocci, there were many organisms with very small capsules and some with none at all; and as stated above, we found that phagocytosis *in vitro* began earlier with old cultures than with young ones. When capsule stains were made at intervals after introducing the bacteria into the pleural cavity, it was found that the capsules were well maintained during the first few hours of proliferation, but at a period ranging usually from 6 to 12 hours following the injection the capsules of some of the bacteria became decidedly smaller than those of the original culture, and in some instances were reduced to minute dimensions or lost completely. With the passing of time, more and more organisms were found with deteriorated capsules or none at all. (Figures 8 to 13). The beginning of phagocytosis coincides with the occurrence of these capsular alterations, and the degree of phagocytosis at any period parallels well enough the degree to which capsular loss is evident.

In addition to uniform shrinkage of the capsule progressing to

complete loss, various other capsular abnormalities make their appearance some hours after infection. The capsules of some of the bacteria swell greatly and the enclosed organisms occupy an eccentric position near the edge. In other cases the capsule becomes irregular in outline. Finally, the entire capsule may be lost at one end of a short chain and preserved at the other end (Figure 13).

As far as we have been able to determine, well encapsulated organisms are not ingested by the phagocytes. We have, however, occasionally seen one or two cocci of a short chain engulfed within a leucocyte while the remainder of the chain projects well encapsulated. Such instances appear to represent the phagocytosis of the part of a chain that has become decapsulated as described above. No capsules were visible about cocci in the interior of leucocytes.

While the above described capsular changes are striking and become steadily more so with the passing of time once they have made their appearance, they never affect all of the organisms simultaneously. At all periods after infection, as long as the pneumococci persist in the exudate in numbers large enough to permit their being found in smears, encapsulated forms occur. Indeed, the extracellular encapsulated forms have always greatly outnumbered the non-encapsulated ones—apparently for the reason that the latter are readily taken up by the leucocytes. It is perfectly clear from these studies that there is no period at which capsular changes suddenly become apparent in all of the organisms of the exudate, but rather that some individual organisms suffer loss of their capsule, while others, side by side, remain unaffected (Fig. 12). Gradually the change affects more and more bacteria, and more and more are found within leucocytes, until complete sterilization has occurred. Plates made of the exudates at intervals up to the point of sterilization have for the most part shown only mucoid colonies composed of capsulated forms. In only two or three instances have we recovered rough colonies from the exudates. This may be merely a reflection of the relative scarcity of decapsulated forms present extracellularly, for we have not systematically made a large number of plates at each interval in the attempt to isolate the rough forms; it may possibly mean that some of the organisms which show no detectable capsule in the smears may actually possess a thin film which regenerates during proliferation

under more favorable growth conditions *in vitro*; or it may indicate the extracellular death of some of the decapsulated bacteria. That this latter circumstance occurs there can be no doubt, for it is not unusual to encounter in the smears decapsulated bacteria which take the stain irregularly and have a shrunken, frayed, degenerated appearance as though undergoing lysis. Similar changes are seen in some of the bacteria about which capsules are still stainable.

The capsular changes described above were regularly observed to occur in all seven of our strains of Type III pneumococci to which rabbits are natively resistant. They occurred *in vivo* to a lesser degree in the rabbit-virulent SV strain, and to no appreciable degree in the Type I pneumococcus. In the case of these latter pneumococci (SV and Type I) that maintained their capsules, phagocytosis was negligible in amount and the animals succumbed to the infection.

Maintenance of the capsule of the Type III pneumococcus in the susceptible mouse. As stated above, all of our strains of Type III pneumococci were virulent for stock white mice. It is therefore interesting to note that all of these strains maintained their capsules in high degree throughout their multiplication in the peritoneal cavity of the mouse. Figure 10 shows the large capsules in the exudate of the mouse 18 hours after the injection of the strain SM which, in the rabbit, showed marked capsular alterations at this period (Figures 9 and 12). Indeed, the average size of the capsules in the mice was, if anything, larger than that of the capsules of the original culture injected. Parallel with the maintenance of the capsule in the body of the mouse up to the death of the animal, there was a complete absence of phagocytosis.

Individual differences in the native resistance of rabbits to Type III pneumococci. Everyone who has studied experimentally the course and pathological effects of any bacterial infection in animals knows well that even when the dosage of bacteria and all environmental conditions are kept as uniform as possible, there are often marked differences between the reaction of different individuals of the same race. Infection of the rabbit with the Type III pneumococcus is no exception to this rule. Different rabbits display quite marked differences in their degree of native resistance to this organism. With the large dose of bacteria used in most of the intracutaneous infec-

tions in the present experiments most animals of a given series will develop lesions of the same general order of severity in the case of a given strain, but the lesions in occasional individuals will be considerably smaller and in others larger and more intensely haemorrhagic. With this dose only extreme differences in resistance are very apparent. With smaller doses that produce at most only relatively small lesions, the individual differences become exaggerated. In the case of the SV rabbit-virulent strain, just as in the case of strains to which the rabbit is resistant, marked differences in individual susceptibility have been observed. Some animals of a given series may survive a given dose that kills most of the animals injected at the same time. Tillett (4), likewise, noted differences in individual susceptibility to this strain. Here, too, if the dose is decreased, the individual differences become much more marked. With highly virulent Type I pneumococci the individual differences in susceptibility are much less obvious, for a very few diplococci will regularly cause the death of rabbits; but even here differences are apparent in the varying length of survival following infection. Individual differences in native resistance appear much more strikingly in the case of bacteria which possess some, but not an extremely high, ability to infect the species in question.

The mechanism of individual differences in native resistance. We have not yet made a careful attempt to determine precisely to what the difference in the native resistance of different rabbits to the Type III pneumococcus is due. Undoubtedly, however, heredity must play a rôle here, just as it has been shown to play a rôle in the case of every infection so far carefully studied experimentally from this standpoint. Numerous investigators have demonstrated clearly that in plants, birds, mice, rats, guinea-pigs and rabbits different degrees of native resistance and susceptibility to a wide variety of bacteria and viruses can be transmitted by heredity (7) (8) (9). The demonstration of this fact, although exceedingly important, does not, however, instruct us as to the mechanism of resistance that is transmitted by heredity. In the case of the rabbit's native resistance to the Type III pneumococcus, the experiments presented here bring to light several highly important mechanisms which operate to bring about the observed resistance. Among these, the fever response of the

animal is the simplest to study from the standpoint of individual resistance. We have not yet completed a thorough study of the matter from this standpoint, but we have repeatedly observed a correlation between the degree of temperature exhibited by infected animals and the rapidity with which the cutaneous lesion regressed, or the pleural infection became sterilized. The height of temperature attained at any one period after infection is no proper measure of the animal's fever response, for the temperature may fluctuate during infection, and the bacteria are able to resume proliferation during periods when the temperature falls below their critical point. All of our evidence indicates that the animals that develop without undue delay and maintain most successfully a continually high temperature will most successfully restrict the infection. This was plainly evident in the animals kept in the incubator room, for here the temperature, though fluctuating somewhat, was constantly maintained at a level considerably above normal, and the lesions were almost always smaller than the second lesions developing in rabbits at room temperature under the influence of the fluctuating fever temperature of an infection on the opposite side of the body. But individual rabbits vary even in the degree to which their temperature becomes elevated in the incubator, and corresponding differences in their ability to restrict an infection induced in the incubator were noted. From these considerations it seems clear that the state of the temperature-regulating mechanism of the individual rabbit will exert a marked effect upon the ability of the animal to resist the infection, and it is not impossible that there may be hereditary differences in this respect although, obviously, the temporary state of the animal resulting from other influences may play a rôle as well. Differences in the degree to which the inflammatory exudates of different animals may tend to induce capsular loss with resulting phagocytosis, independently of temperature, may also play a rôle in determining the relative degree of resistance, but the experiments described above on pleural infection in animals kept in the incubator showed plainly that, in the animal body, fever temperature not only inhibited the growth of the bacteria, but also influenced the rapidity of capsular loss.

What has been said in the preceding paragraph applies only to the

seven strains to which the rabbit is natively resistant. Since the SV rabbit-virulent strain is not so sensitive to the rabbit's fever temperature, the individual variation in the susceptibility to this strain may perhaps be due to a greater power of the fluids of some rabbits to inhibit its growth or induce capsular deterioration. At present, however, there is no information whatever regarding these possibilities, and it may be stated that, not only in the case of this particular strain, but in bacterial infections in general, the mechanism of the resistance of some individuals to bacteria that are virulent for most individuals of the species remains obscure.

DISCUSSION

The studies presented in this paper render understandable the heretofore obscure phenomenon of the native immunity of the rabbit to most strains of Type III pneumococci. This is not one of the forms of native immunity that result from a complete incompatibility of the normal body of the host with the survival of the bacterium; nor is it one of the familiar instances in which immunity results from the immediate susceptibility of the bacteria to the activity of the phagocytic defense of the host. On the contrary, the bacteria in the form in which they enter the tissues cannot be ingested by the animal's phagocytes, and instead of encountering an environment immediately unfavorable to their life, they proliferate markedly for a time after entering the tissues. It is only after a lapse of time that the body of the host becomes a highly unfavorable environment for these bacteria, and it is to the alterations that occur both in the host and in the bacteria after this lapse of time that the observed immunity is due.

This general situation is not peculiar to this particular instance of native immunity. A similar situation exists in native immunity to the tubercle bacillus, although the mechanism in play in that instance is not yet well understood. The rabbit, for example, although highly susceptible to the bovine tubercle bacillus, possesses a high degree of native resistance to the human type. Injected into the body of the rabbit, however, the human type bacillus does not encounter at once an environment incompatible with its survival. On the contrary, it is easy to observe that the bacillus multiplies steadily for a period

of days. Indeed, the studies of Lurie (10) indicate that the human type bacillus multiplies during the first week even more rapidly than does the bovine type to which the animal is highly susceptible. But here, too, as in the native immunity to the Type III pneumococcus, some change takes place after a time in the host-parasite relationship which renders the originally favorable environment of the host an unfavorable one for the human type bacilli, and their proliferation thereafter ceases, most if not all of the bacilli die, and the host survives. The bovine type bacillus, in contrast, continues its proliferation up to the death of the host. In less acute infections such as tuberculosis, it is perfectly plain that specific acquired resistance soon enters the picture, and the degree to which it appears may influence profoundly what, on the surface, might be regarded simply as native immunity. In an acute infection in a natively resistant animal in which the proliferation of the bacteria is arrested shortly after their entry into the tissues, such as in Type III pneumococcal infection in the rabbit, there is less opportunity for specific acquired immunity to enter to any appreciable degree, although there can be little doubt that it does play an important rôle in the recovery of a more susceptible host from acute infections in which the bacteria proliferate for a longer time, and more time is provided for effective antibody formation. In the instance of native immunity under consideration, no participation of specific acquired immunity could be detected. Nevertheless, this instance of native immunity is largely due to the development of a non-specific acquired resistance, conferred by the fever temperature of the rabbit.

The studies reported here show clearly that, shortly after infection, changes occur both in the host and in the bacteria which are demonstrably responsible for the cessation of growth and the ultimate death and disposal of the microörganisms. The alteration of the host that takes place within a number of hours after infection creates in this remarkably short time a condition of greatly enhanced resistance to further infection with fresh bacteria. This enhanced resistance is due to the development of fever, for the degree of temperature with which the rabbit responds to this infection is, as we have shown both *in vitro* and *in vivo*, markedly inhibitory to all of our strains of Type III pneumococci to which the rabbit is resistant. Some hours

after infection there also occurs a change in the bacteria (*i.e.*, a loss of capsule) which renders them phagocytizable. The importance of both of these changes in determining the resistance of the rabbit to these organisms is obvious, and is further indicated by the fact that the only one of the strains to which rabbits were susceptible (SV) was resistant to the degree of heat represented by the rabbit's fever temperature and exhibited much less tendency to lose its capsule and become phagocytizable. The same was true in even a more marked degree of Type I pneumococci that were more highly virulent for the rabbit. Furthermore, our stock white mice were susceptible to the seven strains of Type III pneumococcus to which rabbits were resistant, and the normal temperature of mice is several degrees lower than that of rabbits and does not rise to the level critical for the Type III pneumococcus during infection with this organism. The bacteria proliferate in the mouse, therefore, uninhibited by temperature and, furthermore, preserve their capsules in high degree and resist phagocytosis.

From the indirect evidence that immunization with Type III pneumococci fails, in most cases, to stimulate the production of type-specific antibody in rabbits, and since, as is well known, it is the capsular material of the pneumococcus that incites the production of type-specific antibody, Tillett (4) suggested several years ago that the rabbit may possess some mechanism that damages the capsule of this bacterium. The present studies show clearly that capsular damage does occur in all of the rabbit-resistant strains examined. The cause of the loss of the capsule in the rabbit is not entirely clear. It is clear enough, however, that the normal body of the rabbit is not incompatible with the development of capsules, for during the multiplication of the bacteria in the pre-febrile hours myriads of well-encapsulated organisms are formed. It is also clear that at no period following infection are the capsules of all of the bacteria attacked simultaneously, for at every stage one finds encapsulated organisms by the side of partially or completely non-encapsulated ones. That the capsular deterioration becomes prominent in a variety of media *in vitro* as the cultures increase in age (18 to 24 hours) instructs us that the ability to cause the change is not peculiar to the environment of the rabbit's body or to the rabbit's body fluids. Indeed,

repeated transfers in defibrinated rabbit's blood have not led to the production of non-encapsulated strains. In rabbits with temperatures elevated to fever levels in the incubator before infection, the capsular alterations appear earlier, indicating that the fever temperature not only inhibits the growth of the bacteria but also interferes with the development or persistence of their capsules.

Since the capsular changes make their appearance *in vitro* in older cultures, and are not present to any appreciable degree in young cultures, and since they appear earlier and progress more rapidly in rabbits with temperatures elevated before infection, it appears that the changes tend to occur when the bacteria are subjected to various conditions unfavorable to their growth. It is probable that the inflammatory exudate in which they lie in the body may, itself, become an increasingly unfavorable medium, just as is the case *in vitro* with media in which bacteria grow for a period of time. The fact that the cutaneous site at which the bacteria are injected tends to become sterilized earlier than the spreading edge of the lesion suggests that the local conditions in the older, rather walled off portions of the lesion where the bacteria have proliferated for a longer time are less favorable than are those at the advancing margin where the drifting bacteria have access to a fresher supply of oedema fluid, in which they are clearly able to proliferate more freely. At all events, the capsule of the Type III pneumococcus is decidedly less stable, both *in vitro* and *in vivo*, than is that of the Type I, and the evidence indicates that unfavorable growth conditions of various types tend to cause capsular loss, and that in the rabbit the loss is not a result of a specific capsule-destroying mechanism. We have spoken here of "capsular loss" and "capsular damage" but it is at present impossible to say to what degree in the infected rabbit non-encapsulated organisms represent bacteria which have lost their capsules, or bacteria which, proliferating under unfavorable conditions, failed to form capsules. It is possible that both of these circumstances occur.

The late appearance of phagocytosis of the Type III pneumococcus, both *in vitro* and *in vivo*, is clearly a result of the gradual appearance of non-encapsulated phagocytizable forms which are not present in any appreciable numbers in young cultures. The fact that the phagocytosis of these organisms becomes detectable only after some hours,

and sometimes not to any appreciable degree before 12 to 18 hours, renders desirable a closer examination of any supposed instance of immunity in the absence of phagocytosis, when the phagocytic test has been only the usual 15 min.-1 hr. one. While phagocytosis of the Type III pneumococcus occurs eventually in marked degree in the rabbit, it is, however, no less clear from stained smears of the exudates that extracellular death and disintegration of both encapsulated and non-encapsulated bacteria take place as well. It is of course impossible to form an opinion as to how many of the bacteria found within leucocytes were alive at the time they were ingested, but since the immediate phagocytosis of living non-encapsulated pneumococci can be readily demonstrated, it is altogether likely that here, as in other instances of immunity, phagocytosis plays an important rôle in the active destruction of the non-encapsulated forms. In studies to be published elsewhere we have found that non-encapsulated, rough pneumococci, which are quite avirulent for normal rabbits because of the rapidity with which they are phagocytized, will grow freely and produce septicaemia in rabbits deprived of leucocytes.

Finally, a circumstance contributing undoubtedly to the native resistance of the rabbit to Type III pneumococci is the fact that these organisms proliferate much less rapidly in the rabbit even during the pre-febrile period than does the highly virulent Type I. This is true likewise of the growth of these organisms *in vitro* in rabbit serum or defibrinated rabbit blood whether at 37° or 40°–41°C. This more sluggish growth in the rabbit's fluids prevents the formation of overwhelming numbers during the period before the inhibiting fever is well developed.

The striking deleterious effect of temperatures of 104°–106°F. upon the proliferation and survival of these seven strains of Type III pneumococci suggests, obviously, the possibility that Type III infection in the human being may be favorably influenced by maintaining the temperature within this range by artificial means, and this possibility we shall endeavor to test. We have not yet made a systematic study of the degree of temperature exhibited by patients with Type III infection, but the records of the last three fatal cases of Type III lobar pneumonia in this hospital show that in none did

the temperature reach 104°—the critical temperature of this organism. It must be remembered, however, that the deleterious effect of 104°–106°F. upon the Type III pneumococcus is not an immediate one that attacks all of the organisms simultaneously. It requires time; and a fluctuating temperature, even though it rises to the critical level, will, when it falls below that level, permit the growth of any surviving organisms. The maintenance of a continually high level of temperature by artificial means will obviously be more destructive to the bacteria. We have studied only eight strains of Type III, but since seven of the eight were sensitive to this degree of temperature, although isolated from different human sources at widely different times, it appears reasonable to believe that most strains of Type III are probably susceptible to this temperature. Whether the destruction of the bacteria by temperature can be effected as readily in the human being as in the rabbit or in the test tube is, of course, impossible to foretell, but the observations recorded here at least render the attempt desirable.

CONCLUSIONS

1. The native immunity exhibited by the rabbit to most encapsulated, mouse-virulent strains of Type III pneumococci is characterized by the fact that the bacteria proliferate progressively in the tissues for some hours causing, with the dose here used, a local lesion of considerable severity, often accompanied by bacteraemia. The proliferation of the bacteria is then checked, they are destroyed, and the animal recovers completely.

2. The proliferation of the Type III pneumococcus in the natively resistant rabbit is distinctly less rapid than that of virulent Type I strains, to which the rabbit is highly susceptible; but this relatively slower growth, while playing a definite rôle in the observed immunity, is by no means sufficient to prevent the development of a severe lesion or to account for the survival of the animal.

3. Within the first 24 hours of the infection the rabbit acquires a greatly enhanced resistance to further infection. This resistance is shown to be due not to acquired, specific immunity, but to the fever that develops as a result of the infection. Rabbits with temperatures elevated to 104°–106°F. by keeping them in an incubator before and

during infection develop only relatively slight lesions which rapidly heal; and *in vitro*, strains of Type III pneumococci to which rabbits are natively immune (the great majority of strains in our own experience and in that of others) were found to be markedly sensitive to the degree of heat represented by the rabbit's fever temperature (104°–106°F.).

4. These encapsulated strains to which the rabbit is resistant are not phagocytized promptly either *in vitro* or *in vivo*, but after some hours sojourn in the body of the rabbit they gradually lose their capsules and are then avidly phagocytized. Capsular loss occurs likewise *in vitro* as the cultures become older, and early phagocytosis can be observed to occur when old instead of young cultures are used. Starting with young cultures, phagocytosis may be demonstrated in serum-leucocyte-bacteria mixtures if the observations are continued over a period of hours. The loss of capsule appears to be dependent upon unfavorable conditions, whether of temperature or of surrounding medium. In addition to destruction of the bacteria by phagocytosis, extracellular death of cocci also takes place. The occurrence of both of these processes is accelerated in the rabbit by temperatures of 104°–106°F.

5. As in all instances of native immunity, different individuals differ somewhat in the degree of their resistance. The possible mechanisms underlying these individual differences in the present instance are discussed.

6. The resistance of a given rabbit to a given dose of Type III pneumococci will depend upon the degree to which the various above factors enter the picture following infection.

7. The marked sensitivity of the great majority of our strains of Type III pneumococci to temperatures of 104°–106° *in vitro*, together with the striking inhibitory influence upon the infection that is effected by elevating the rabbit's temperature to this level, renders desirable an investigation of the effect of maintaining at this level the temperature of human beings infected with these organisms.

REFERENCES

(1) Rich, A. R. and McKee, C. M.: *Bull. Johns Hopkins Hosp.*, 1934, **54**, 306.
(2) Rich, A. R.: *ibid.*, 1933, **52**, 203.
(3) Singer, E. and Adler, H.: *Z. f. Immunitätsforsch.*, 1924, **41**, 71, 468.

(4) TILLETT, W. S.: *J. Exp. Med.*, 1927, **45**, 1093.
(5) RICH, A. R. AND McKEE, C. M.: *Bull. Johns Hopkins Hosp.*, 1934, **54**, 461.
(6) RICH, A. R.: *Jour. Immunol.*, 1936, **30**, 389.
(7) WRIGHT, S. AND LEWIS, P. A.: *Am. Naturalist*, 1921, **55**, 20.
(8) IRWIN, M. R.: *Genetics*, 1932, **17**, 203.
(9) WEBSTER, L. T.: *J. Exp. Med.*, 1933, **57**, 793, 819.
(10) LURIE, M. B.: *ibid.*, 1928, **48**, 155.

EXPLANATION OF ILLUSTRATIONS

PLATE 1

FIG. 1. Typical lesion with haemorrhage in previously normal rabbit 48 hours after intracutaneous injection of 0.1 cc. of a 1–10 dilution of a 7-hour culture of Type III pneumococci (strain SM). The lesion always regresses and the rabbit survives.

FIG. 2. 48-hour-old lesion in rabbit that was infected, on the side of the body illustrated, with the same dose of the same strain at the same time as for rabbit in Fig. 1. The illustrated infection was made, however, when there was a 24-hour-old lesion of first infection on the opposite side of the body. The illustrated lesion was never larger at any time and disappeared earlier than the lesion of first infection.

FIG. 3. 48-hour-old lesion produced by same dose of same strain as in Figures 1 and 2, but in this case the rabbit's temperature was elevated to 105°–105.5° by sojourn in incubator for 12 hours previous to infection and following infection. The lesion was never larger at any time. Almost no oedema; no haemorrhage; prompt regression.

FIGS. 4 AND 5. Leucocytes from pleural exudate of rabbit 18 hours after injection of 1.0 cc. of a 7-hour culture of strain SM. Abundant phagocytosis. Intact and partly digested cocci.

FIG. 6. *In vitro* phagocytosis of strain RM in rabbit serum-leucocyte mixture at end of 8 hours.

FIG. 7. Leucocytes from pleural exudate of rabbit 12 hours after injection of 1.0 cc. of a 7-hour culture of strain O. Abundant phagocytosis.

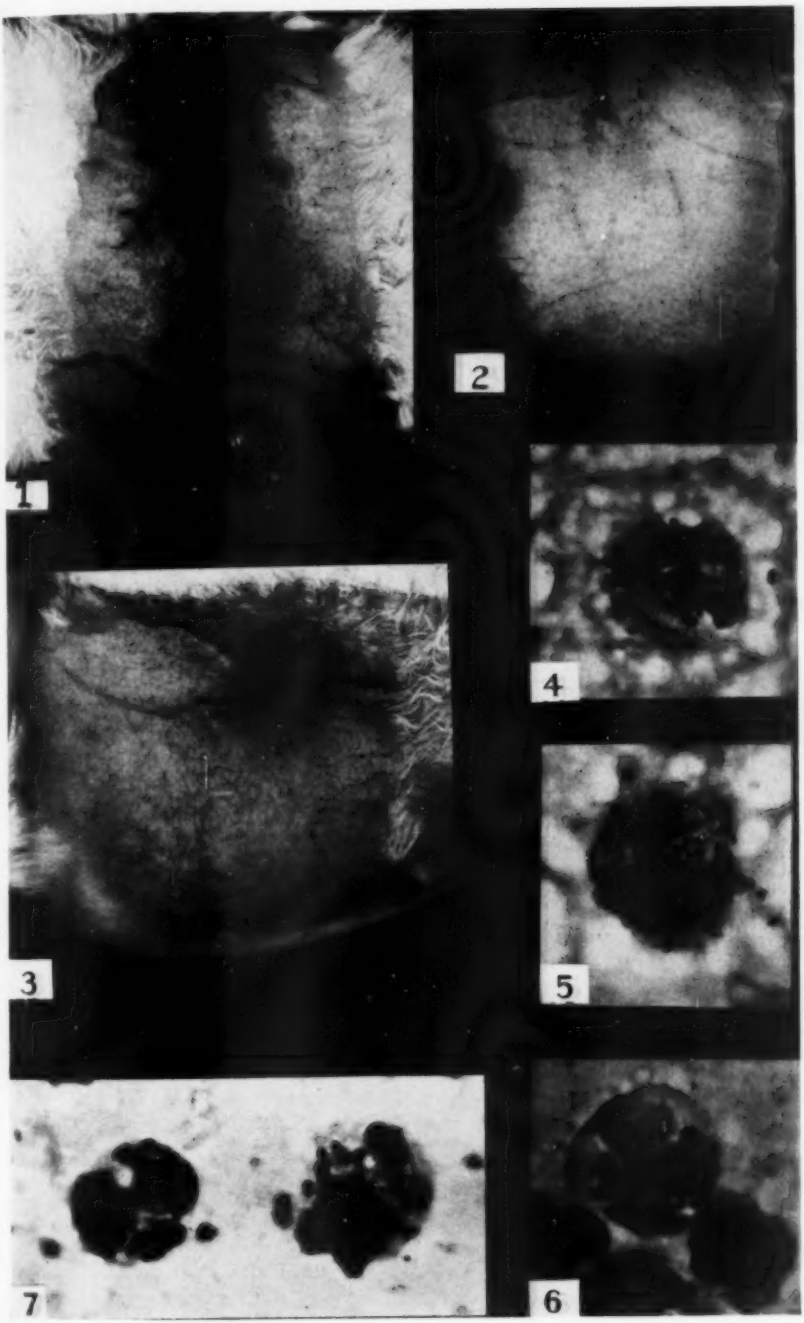

1
2
3
4
5
6
7

PLATE 2

FIG. 8. Relief stain of exudate showing appearance of capsules 4 hours after injection of 1.0 cc. of a 7-hour culture of strain SM intrapleurally, in rabbit. The bacteria have multiplied greatly and have well formed capsules.

FIG. 9. Relief stain of exudate showing two cocci with very slight or no capsules 18 hours after injection of 1.0 cc. of a 7-hour culture of strain SM intrapleurally, in rabbit. Most of the free bacteria have disappeared from the exudate. The phagocytosis shown in Figures 4 and 5 is from the same rabbit at the same time.

FIG. 10. Large perfect capsules of strain SM in peritoneal exudate of mouse 18 hours after infection. Compare with Figures 9, 12 and 13. All same magnification.

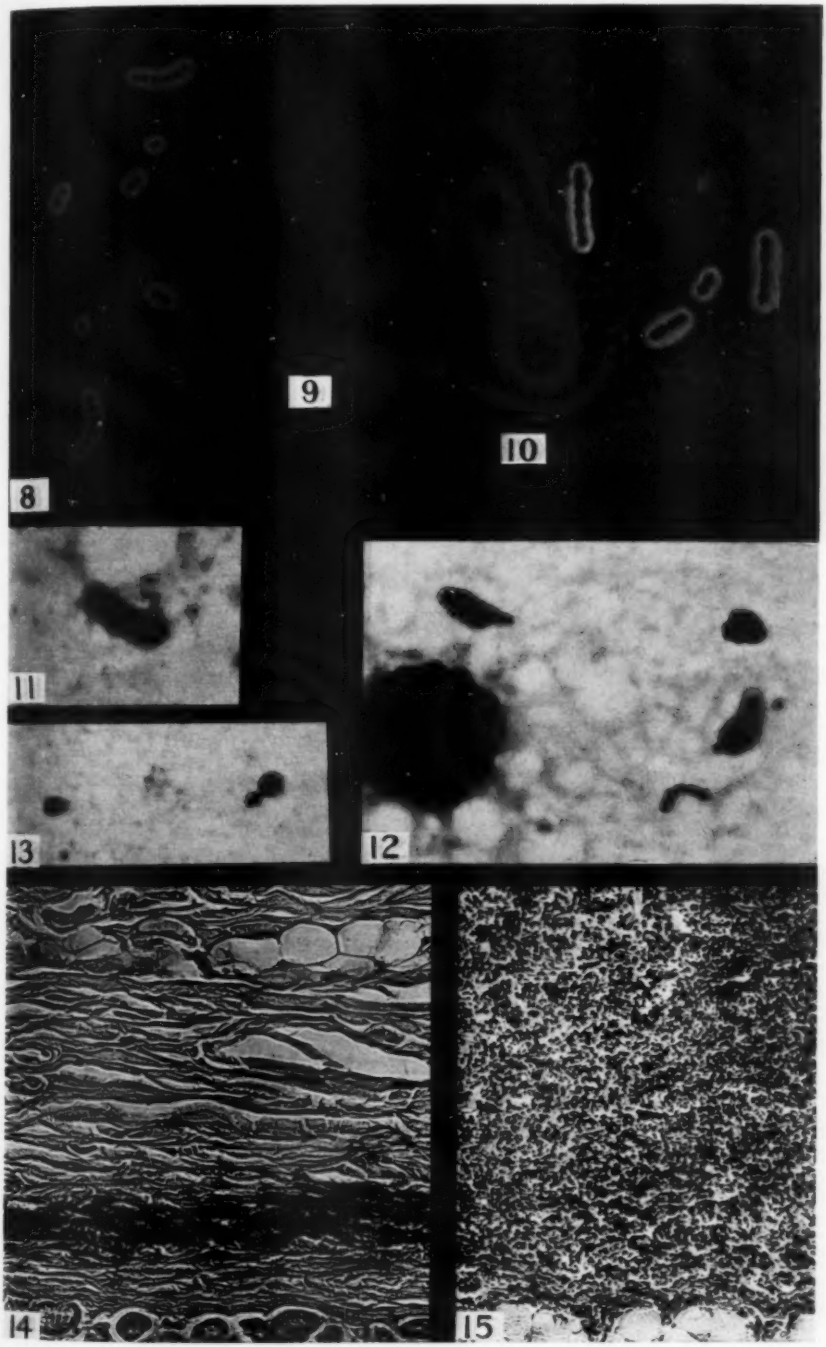
FIG. 11. Average appearance of capsules in direct stain, 5 hours after injection of 1.0 cc. of a 7-hour culture of strain SM intrapleurally, in rabbit. Compare with Figure 13 which is of same magnification.

FIG. 12. Direct capsule stain 18 hours after injection of 1.0 cc. of a 7-hour culture of strain SM intrapleurally, in rabbit. Shows two chains with incomplete capsules and one with no capsule at all.

FIG. 13. Direct capsule stain 5 hours after injection of 1.0 cc. of a 7-hour culture of strain SM intrapleurally in rabbit with temperature elevated to 105°–106° before and during infection by sojourn in incubator. Chain at right shows partial loss of capsule. Capsules are also much smaller than in control infected at same time and kept at room temperature (Fig. 11).

FIG. 14. Site of cutaneous injection of 0.1 cc. of a 1–1000 dilution of strain O in a benzol-treated leucopenic rabbit at time of death with septicaemia, 48 hours after infection. The thick black line is a solid mass of pneumococci. No leucocytic emigration. Low magnification. Details of benzol treatment given in reference (1).

FIG. 15. Site of infection in control rabbit injected with same dose at same time as the rabbit in Figure 14, and killed at 48 hours. Blood culture sterile. Heavy leucocytic infiltration. Same magnification as Fig. 14. Cocci are extremely rare and can be found only on search with higher magnification.



UNIV. OF MICH.

BOOK REVIEWS

Community Hygiene. By LAURENCE B. CHENOWETH, M.D. and WHITELAW REID MORRISON, M.D. Pp. 317. $2.50. (*F. S. Crofts & Co., New York, 1934.*)

This somewhat haltingly written textbook is offered for use in college courses in hygiene. The authors state that their lecture-notes in the course, presented at the University of Cincinnati and at Oberlin College, "were revised and rewritten until it seemed that the students were becoming intelligent regarding the subject," and at this point the book was written.

The broad field of community hygiene is covered fairly adequately in this volume, in very much the same fashion as is to be found in numerous other similar textbooks, but evidently the material has not been very well digested by the writers and the presentation suffers from a race to crowd in as many facts per paragraph as possible. At times, a curiously not-quite-right use of a word, as in the definition (p. 54) "Epidemic diseases are those which break out suddenly, run an epidemiological course, and then become endemic," makes one doubt the authors' familiarity with the subject, while statements such as "Lack of sunshine predisposes to tuberculosis" (p. 52) are certainly more positive than existing knowledge would justify. The following sentence (p. 50): "It is quite obvious from the proportions of human milk that in general there are several defects in the diet of most people: (1) too high a proportion of protein, (2) not enough water, (3) no thought as to vitamins," is a classic blend of poor writing and loose thinking. However, the book has many excellent charts and tables.

M. L. L.

The Woman Asks the Doctor. By EMIL NOVAK. 189 pp. $1.50. (*Williams & Wilkins Co., Baltimore, 1935.*)

"The Woman Asks the Doctor" is a small volume of less than 200 pages in which the author talks "collectively to many women as every physician and gynecologist must often, in his consulting room, talk to one woman." The author's comprehensive knowledge of the literature and his ability to impart it to others rightfully gives to him the authorship of such a work. Liberal space is devoted to a simple exposition of the physiology of the female functions. The scientific facts and theories are presented in such a way that they may be easily understood by the woman of average intelligence and education. The author states in the preface that he intends to discuss those problems which in his experience are of most interest to women. No two gynecologists have exactly the same experience but probably the questions which "the woman asks the doctor" most frequently have to do with sterility and birth control. One group of women earnestly desire children and cannot become pregnant. Another group, and a

much larger one, just as ardently wish to know how to keep from having too many children, so that they may give to those which they already have the benefits that should belong to every human being. The question of sterility is quite simply and adequately discussed. On contraception the author seems to avoid the question, beyond speaking at some length of the "safe period." The other methods of birth control recognized as being more effective are not discussed.

The chapter which is probably of greatest value to womanhood is the one on cancer. In it the author emphasizes the early signs and symptoms which should make the woman cancer conscious. The education of the medical profession concerning the importance of early symptoms of cancer has been most discouraging. Every gynecologist of experience sees many cases of advanced carcinoma for every early case. Often the woman has consulted her family physician for weeks or months before a pelvic examination or satisfactory examination of her breasts has been suggested. Novak attempts to go directly to the woman and to make her sufficiently intelligent on the subject to demand the necessary examination. If the book teaches her to do so, it will do a good work.

R. W. TEL.

The Science and Art of Nursing. By ELLA L. ROTHWEILER, R.N. 1196 pp. illus. $3.00. (*F. A. Davis Co., Phila., 1935.*)

This 1196-page volume introduces a new and amazingly comprehensive type of text into the field of nursing. "Based on the acceptance of pedagogical principles, taking into consideration something of the philosophy of Education and the psychology of learning processes," the book is well planned for the student nurse. Each chapter includes a valuable list of 'review questions' and suggested projects.

The author has given a great deal of space to the mental and spiritual sides of nursing. This phase has usually been avoided in print, and yet there is a definite need for it. How many nurses will feel that in several places this book borders on sentimentality?

This text will probably be found most useful in smaller schools where one busy instructor is expected to teach and correlate the practice and theory of nursing.

E. J. H.

The Treatment of Diabetes Mellitus. By ELLIOTT P. JOSLIN. 1935. 5th Ed. 620 pp. illus. $6.00. (*Lea and Febiger, Philadelphia, Pa.*)

A widely revised edition of the well known compendium on diabetes. The revision is amazingly complete and up to date. The author describes with great care some of the more modern concepts of the pathology and treatment of the disease. Where a theory remains as yet unproved, he states the facts which are known, clearly and concisely, and then from his vast experience gives the reader the reasons for his own present policy in regard to them.

The work can be very highly recommended as a text and reference book on diabetes mellitus for students and practitioners.

J. E. H.

Injuries and Their Treatment. By W. ELDON TUCKER. 173 pp., 80 illus., $3.75. (*Oxford University Press, New York, 1935.*)

This book deals with the treatment of soft structure injuries, with emphasis on early but discriminating mobilization directed towards dispersion of the excess of traumatic effusion. Such therapy will be open to criticism, as the author readily admits, since it is the common belief that the object of this traumatic effusion is for repair, and that a factor essential to recovery should therefore not be thus removed.

However, the author states that much of this effusion is excessive, makes for adhesions and thickenings, impedes the process of repair, and prevents the formation of a good scar.

Two main types of inflammation are described, (1) Bacteriological, and (2) Non-bacteriological. The first requires absolute rest of the part affected, whereas in the non-bacteriological type, of which traumatic inflammation is an important heading, rest may be contra-indicated.

The rationale of treatment is:

(1) Absolute rest is wrong in treatment of most injuries to joints and muscles.

(2) Every endeavor must be made to disperse the excess of traumatic effusion. If possible, this should be aspirated, as it may save weeks of disability and prevent the risk of its becoming infected. At this stage, the traumatic effusion is fluid and the fibrin and cells have not had time to be deposited as adhesions and thickening. Therefore, if aspiration is carried out, these complications have no chance of occurring.

(3) The control of pain in the treatment of injuries must be carefully considered, as in the early stages it may be severe.

Different methods are prescribed for different injuries, varying from absolute rest to active mobilization under an anesthetic.

The author is himself a well-known athlete, who has specialized in traumatic injuries incurred in sports. His teachings advance the ideas of Sir James Paget, who as long ago as 1868, wrote an article on "Cases that Bone-setters Cure." It is well to bear in mind, however, the teaching of Hamilton Russell that in cases of fractures involving, or in the immediate vicinity of a joint—"When great stiffness is present, in spite of the surgeon's efforts to increase the mobility by passive movements, such stiffness will persist so long as the surgeon persists. When the surgeon abandons the case in dispair, improvement will at once commence."

I. R. T.

The Early Diagnosis of the Acute Abdomen. By ZACHARY COPE. 7th ed. 254 pp., illus., $3.75. (*Oxford Press, New York, 1935.*)

Hawklike and constant observation of a patient who may have an acute abdominal condition so that early surgical intervention may be possible is the keynote of these chapters. "The general rule can be laid down that the majority

of severe abdominal pains which ensue in patients who have been previously fairly well, and which last as long as six hours, are caused by conditions of surgical import."

Emphasis is put on anatomical knowledge in interpreting the possible pathological process. Because the diaphragm begins to develop in the region of the fourth cervical segment, an inflammatory lesion of the upper abdominal cavity, such as a perforated peptic ulcer, may cause pain or hyperesthesia of the skin in the supra- and infra-clavicular fossae. An inflamed appendix lying on the fascia of the obturator internus muscle will cause pain if the muscle be put through its full movement by rotating the flexed thigh inward to its extreme limit, the pain being referred to the hypogastrium.

Chapters follow describing in detail the more important acute conditions of the abdomen and their differential diagnosis.

Chief additions to this seventh edition of this excellent book are paragraphs showing the help which may sometimes be obtained from x-ray examination in the diagnosis of intestinal obstruction.

I. R. T.

Human Pathology. A text-book. By HOWARD T. KARSNER. 4th ed., 1013 pp. illus., $10.00. (*J. B. Lippincott Co., Philadelphia, 1935.*)

In this fourth edition many subjects have undergone extensive revision, and many new references to the literature have been added. The section on tumors, as stated in the preface, has been completely rewritten; and the new edition is indeed admirable. The extensive bibliography is especially valuable.

As in the past, the material in the new edition is divided into general and special pathology, which seems to entail frequent repetition. To the reviewer it would seem unfortunate that certain subjects, such as the exanthematous diseases, are not described. The explanation of the differences between haemorrhagic and pale infarcts appears incomplete, since the effects of venous occlusion and of partial arterial occlusion are not taken into account. In the discussion of disturbances of blood circulation distinction is not made between the effects of failure of the right and of the left ventricle of the heart. A fuller discussion of allergy and immunity would be invaluable to the student, but in the description of tuberculosis the importance of allergy in the pathogenesis of the lesions is not suggested. The author says, too, that tuberculous meningitis is probably haematogenous, an idea that has been thoroughly discredited by anatomical and experimental studies. The experimental production of oedema by low protein diets is given as an example of experimental nephrosis, suggesting that lipoid necrosis in man is part of a general "metabolic" disturbance. It is evident, however, that protein deficiency with consequent oedema has no close relation to a disease of the kidney characterized by massive proteinuria and subsequent oedema.

The text is abundantly illustrated by drawings and photographs.

S. B.